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Influence of Diet and Starvation on Toxicity of Endrin to Fathead Minnows (Pimephales Promelas)

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400 33 Goteborg, Sweden

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16. ABSTRACT

Endrin toxicity to fathead minnows, Pimephales promelas, was determined in flow-through toxicity tests at 25°C for periods of up to 30 days.

Three months prior to endrin exposure six groups of fish were fed artificial diets containing an increasing percentage of fat, and two groups were fed either a high or low ration of frozen brine shrimp. Fish fed frozen brine shrimp showed better growth, survival and fewer deformities (hemorrhagic kidney lesions and lordosis) than fish fed artificial diets. Based on 96-hr LC50 values, fish fed diets low in dietary fat were 2.4 times more susceptible to endrin than fish fed frozen brine shrimp. On the same basis (96-hr LC50) fish fed a low ration level of brine shrimp were 2.0 times more susceptible to endrin than fish fed an unrestricted ration level of brine shrimp.

In another series of experiments, adult fathead minnows starved for 80 days were 2.0 times more susceptible to endrin than recently fed fish. Fathead minnows captured in a small pond on April 30 were also about 2.0 times more susceptible than recently fed fish. There was no detectible relationship between body weight and susceptibility of fathead minnows to acute endrin toxicity.

The reduced tolerances of endrin caused by a low percentage of dietary fat, reduced ration level and starvation were correlated to an increased total body water content. The underlying reason is assumed to be a concomitantly decreased total body lipid content.

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INFLUENCE OF DIET AND STARVATION ON TOXICITY OF ENDRIN TO FATHEAD MINNOWS (*PIMEPHALES PROMELAS*)

by

Goran Dave
Department of Zoophysiology
University of Goteborg
Fack
400 33 Goteborg
Sweden

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Project Officer

James M. McKim
U.S. Environmental Protection Agency
Environmental Research Laboratory-Duluth
Duluth, Minnesota 55804

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Duluth, Minnesota 55804

U.S. ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL RESEARCH LABORATORY-DULUTH
6201 CONGDON BOULEVARD
DULUTH, MINNESOTA 55804

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise, and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth contributes to this multidisciplinary focus through programs engaged in

--studies on the effects of environmental contaminants on the biosphere, and

--a search for ways to prevent contamination and to recycle valuable resources.

ABSTRACT

Endrin toxicity to fathead minnows, Pimephales promelas, was determined in flow-through toxicity tests at 25°C for periods up to 30 days.

Three months prior to endrin exposure 6 groups of fish were fed artificial diets containing an increasing percentage of fat, and 2 groups were fed either a high or a low ration of frozen brine shrimp. Fish fed frozen brine shrimp showed better growth, survival, and fewer deformities (hemorrhagic kidneys and lordosis) than fish fed artifical diets. Based on 96-hr LC50-values, fish fed diets low in dietary fat were 2.4 times more susceptible to endrin than fish fed frozen brine shrimp. On the same basis (96-hr LC50) fish fed a low ration level of frozen brine shrimp were 2.0 times more susceptible than fish fed an unrestricted ration level of brine shrimp.

In another series of experiments, adult fathead minnows starved for 80 days were 2.0 times more susceptible to endrin than recently fed fish. Fathead minnows captured in a pond on April 30 were also about 2.0 times more susceptible than recently fed fish. There was no detectable relationship between body weight and suceptibility of fathead minnows to acute endrin toxicity.

The reduced tolerances to endrin caused by a low percentage of dietary fat, reduced ration level and starvation were correlated to an increased total body water content. The underlying reason is assumed to be a concomitantly decreased total body lipid content.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

B.W.	-- body weight
D.W.	-- dry weight
LC50	-- lethal concentration 50%
MST	-- Median Survival Time
w.w.	-- wet weight

SYMBOLS

$CaCO_3$	-- calcium carbonate
H_2O	-- water, used for total body water content in Table E3

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SECTION 1

INTRODUCTION

The nutritional aspects of toxicity studies with aquatic organisms have only recently started to receive the attention of aquatic toxicologists, and the importance of nutritional factors like dietary ingredients, percent composition and ration level on the toxicity of selected toxics has become apparent.

Cairns et al. (1975) found that short-term starvation (0 to 72 hrs) in Orsanco strain goldfish Carassius auratus (Orsanco, 1977) had no significant influence on the acute toxicity of zinc indicating that short-term effects of starvation on acute toxicity (<24 hrs) may be of minor importance. However, the importance of dietary composition on response to toxicants has been demonstrated in several previous studies. Mehrle et al. (1977) found a six-fold difference in the 96-hr LC50's (8.2 to 47 µg/l) of rainbow trout (Salmo gairdneri) when they were fed six different diets for 42 days prior to exposure to chlordane. The underlying reason for these differences in acute toxicity was not elucidated. A four-fold difference in the chronic toxicity of copper to the water flea (Daphnia magna) was found between daphnids fed an algal versus a trout pellet-based diet (Winner et al., 1974). In another study on interactions between dietary levels of vitamin C and exposure to toxaphene in channel catfish (Ictalurus punctatus) (Mayer et al., 1978), it was shown that increased levels of dietary vitamin C counteracted the effects of toxaphene on body weight, collagen content of the vertebrae, incidence of spinal deformities, and histopathological effects on the skin. Furthermore, increased dietary levels of vitamin C increased the activity of liver aromatic hydrocarbon hydroxylase, an enzyme involved in detoxification, and caused a reduction in toxaphene residues. Phillips and Buhler (1979) found that rainbow trout fed two different diets (Oregon Moist Pellets or tubificid worms) at three ration levels for 61 days responded differently in terms of fat elaboration and dieldrin accumulation.

Based on the existing knowledge of the effects of nutrition on the response of aquatic organisms to toxicants, it can be concluded that nutritional factors have definite effects on both acute and chronic toxicity. Therefore, since one of the main objectives of toxicity tests with aquatic organisms is to estimate maximum tolerable concentrations of chemicals, it is important to understand to what extent results of laboratory toxicity tests are influenced by nutritional factors, especially when food supply (amount as well as composition) in nature is often quite different from that in the laboratory.

The purpose of this study was to examine the influence of dietary composition and ration level (including starvation) on the toxicity of a

lipophilic (fat soluble) chemical to the fathead minnow (Pimephales promelas). The chemical used for this purpose was endrin (1,2,3,4,10,10-hexachloro-6,7,epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-end o-5,8-dimethanonaphthalene), which is extremely toxic to fish, [96-hr LC50 from 0.26 µg/l for fathead minnows (Pimephales promelas) to 8.25 µg/l for bluegills (Lepomis macrochirus) (Solon et al., 1969; Katz and Chadwick, 1961)]. Fathead minnows were selected as the fish species for this study based on availability and general importance in aquatic toxicology.

Since a lipophilic toxicant was studied, special emphasis was focused on lipid metabolism. Pretreatment of fish prior to toxicity tests included feeding for three months with diets containing various amounts of lipids as well as specific types of lipids. In order to deplete the lipid depots, various groups of fish were also subjected to starvation for up to three months prior to toxicity tests. In the starvation experiments three sources of fathead minnows were used in order to reveal a possible influence of pretreatment prior to the starvation period. The hypothesis that the total lipid content of a fish is important in its short-term survival when exposed to a lipophilic toxicant like endrin was evaluated. This was measured by a determination of survival time, total body weight, and total body moisture content following endrin exposure. Body moisture content in fish was shown to be inversely proportional to lipid content (Love, 1970).

SECTION 2

CONCLUSIONS

Fathead minnows (Pimephales promelas) fed frozen brine shrimp showed better growth and survival and fewer deformities (hemorrhagic kidneys and lordosis) than those fed recommended reference research diets. In those fish fed only the reference research diets supplemented with either 10% corn oil, salmon oil, or a 1:1 mixture of these oils no differences were observed in survival, growth or number of deformities.

Acute toxicity of endrin was increased 2.4 times by a lowered percentage of dietary fat, 2.0 times by a restricted ration level, and approximately 2 times following starvation for 80 days prior to exposure. Fathead minnows trapped in a pond in the spring (April 30) were approximately 2 times more susceptible to endrin than the laboratory stock. The higher susceptibility in wild minnows reflected their poor nutritional status in the spring.

An increased susceptibility to endrin in fathead minnows was positively correlated to an increased total body water content, reflecting a lowered level of total body fat. Re-localization of endrin to non-target tissues like adipose tissue seems to be an important mechanism for resistance to endrin. A dose-dependent reduction in total body water content of fathead minnows exposed to endrin was also observed.

There was no detectable relationship between body weight and susceptibility of fathead minnows to acute endrin toxicity.

SECTION 3

RECOMMENDATIONS

Reference research diets should not be used for the culture of fathead minnows (Pimephales promelas) since they result in an increased mortality, a greater incidence of deformities (hemorrhagic kidneys and lordosis) and decreased growth compared to fish fed frozen brine shrimp (Artemia salina).

A lowered level of dietary fat, a lowered ration level and long-term starvation prior to toxicity tests with a lipophilic chemical such as endrin can produce significantly lower LC50 values. This should be considered in standardized toxicity tests with this type of chemical.

No influence of acclimation to test temperature from 2 to 23 days was evident on the acute toxicity of endrin in wild fathead minnows. On the other hand, feeding for 1 week prior to exposure made the wild minnows more resistant to endrin. Therefore, a more detailed study on differences in susceptibility to lipophilic chemicals between laboratory fish and field-collected fish on a seasonal basis should be made. Such a study should preferably be complemented by measurements of condition factor, water content and lipid content for different groups of fish, in order to achieve a better understanding of the variations in susceptibility of different lots of fish.

Considering the findings in the present study, acclimation to test conditions for 1 week without feeding in both laboratory stock fish and wild fish seems to be a sound basis for the comparison of 96-hr LC50 values.

SECTION 4

MATERIALS AND METHODS

SOURCES OF EXPERIMENTAL FISH

Two sources of fathead minnows were used in this experiment. One was the laboratory brood stock of the Environmental Research Laboratory-Duluth, Minnesota, and the other was a private pond owned by one of the laboratory staff members. This artificial pond (surface area: 18 acres; maximum depth: 1.6 m), supplied with water from a small spring fed stream was, after its construction in 1972, stocked with fathead minnows from the Duluth laboratory brood stock in 1973. Since then, this population of fathead minnows has gone through approximately 5 generations. Therefore, these fish have a natural (wild) background in terms of diet (pond organisms), seasonal variation, and natural reproduction.

For the diet experiments, fathead minnow larvae hatched December 8-22, 1978 were used. After hatching, these larvae were fed live newly hatched brine shrimp (Living World, San Francisco Bay Brand), and later supplemented with frozen brine shrimp (San Francisco Bay Brand, Newark, California 94560; Stock #65006). On January 22 and 23, 1979 one-month-old fish were randomly divided into seven groups of 220 fish each. After determination of total body weight (ten fish at a time) in a tarred aluminum foil cup containing approximately 20 ml water, they were transferred to pretreatment tanks. Experimental groups of fish in this study were designated by numbers I through VIII (Table 1).

For the starvation experiments adult (more than five months old) fathead minnows from the laboratory stock were divided into 4 groups of 50 fish each on January 25, March 2, March 23, and April 9, 1979. Individual body weights of fish were obtained by weighing each fish in a glass beaker containing approximately 300 ml of water and designated numbers IX through XII. A fifth group of >50 fish from this source (not weighed before the toxicity test) were netted on April 30 and fasted 24 hrs prior to the toxicity test (group XIII, Table 2).

In addition, two groups of pond fish (60 fish each) held in the laboratory since September 1978 at 10°C and fed Trout Starter granules, SD 7-30 (78) (Glenco Mills, Inc., Glenco, Minnesota 55336) once daily Monday through Friday were selected for starvation experiments on March 8, 1979. After determination of individual body weights as described above, this group was put into one of the pretreatment tanks and designated number XIV. Another group of 120 pond fish from the same source and held in the laboratory under the same conditions were netted and put into one of the pretreatment tanks on April 30, 24 hrs prior to toxicity test (group XV, Table 2).

TABLE 1. SUMMARY OF EXPERIMENTAL EXPOSURES TO ENDRIN WITH FATHEAD MINNOWS FED DIFFERENT DIETS PRIOR TO EXPOSURE

Group of fish	Experiment no.[a]	Dietary pretreatment	Exposure to Endrin	
			Conc. no.[b]	Period of exposure
		ARTIFICIAL DIETS		
I_1	1	Diet I for 78 days	1,2,3,4,5	- 29 days
I_2	2	Diet I for 92 days	1,2,3	- 15 days
I_{3a}	3	Diet I for 99 days	1,2	- 7 days
I_{3b}	3	Diet I for 92 days plus Diet VIII for 7 days	1,2	- 7 days
II_1	1	Diet II for 78 days	1,2,3,4,5	- 29 days
II_2	2	Diet II for 92 days	1,2,3	- 15 days
III_1	1	Diet III for 78 days	1,2,3,4,5	- 29 days
III_2	2	Diet III for 92 days	1,2,3	- 15 days
IV_1	1	Diet IV for 78 days	1,2,3,4,5	- 29 days
IV_2	2	Diet IV for 92 days	1,2,3	- 15 days
V_1	1	Diet V for 78 days	1,2,3,4,5	- 29 days
V_2	2	Diet V for 92 days	1,2,3	- 15 days
VI_1	1	Diet VI for 78 days	1,2,3,4,5	- 29 days
VI_2	2	Diet VI for 92 days	1,2,3	- 15 days
		BRINE SHRIMP DIETS		
VII_1	1	Diet VII for 78 days (high ration)	1,2,3,4,5	- 29 days
VII_2	2	Diet VII for 92 days (high ration)	1,2,3	- 15 days
$VIII_1$	1	Diet VIII for 78 days (low ration)	1,2,3,4,5	- 29 days
$VIII_2$	2	Diet VIII for 92 days (low ration)	1,2,3	- 15 days
$VIII_3$	3	Diet VIII for 100 days (low ration)	1,2	- 7 days
$VIII_4$	4	Diet VIII for 86 days (low ration)	1	- 3 days

[a] Experiments 1, 2, 3 and 4 were started April 30, May 14, May 22, and May 8, respectively.

[b] Concentration No. 1 was the highest one followed by 1:1 dilutions producing nominal concentrations of 1/2, 1/4, 1/8 and 1/16 of this concentration. Concentration No. 6 (not presented above) was the control.

TABLE 2. SUMMARY OF EXPERIMENTAL EXPOSURES TO ENDRIN WITH FATHEAD MINNOWS STARVED FOR VARIOUS PERIODS PRIOR TO EXPOSURE

Group of fish	Experiment no.[a]	Source	Starvation prior pretreatment	Exposure to Endrin	
				Conc. no.[b]	Period of exposure
IX	1	Laboratory fish	95 days	1,2,3,4,5	- 29 days
X	1	Laboratory fish	59 days	1,2,3,4,5	- 29 days
XI	1	Laboratory fish	38 days	1,2,3,4,5	- 29 days
XII	1	Laboratory fish	21 days	1,2,3,4,5	- 29 days
XIII	1	Laboratory fish	24 hrs	1,2,3,4,5	- 29 days
XIV	1	Pond fish fed in the laboratory during winter	53 days	1,2,3,4,5	- 29 days
XV_1	1[c]	Pond fish fed in the laboratory during winter	24 hrs	1,2,3,4,5	- 29 days
XVI_1	1[c]	Pond fish captured just prior to test	24 hrs	1,2,3,4,5	- 29 days
XV_2	2	Pond fish fed in the laboratory during winter	14 days	1,2	- 4 days
XVI_2	2	Pond fish captured and acclimated for 14 days	14 days	1,2	- 2 days
XV_3	3	Group XV_2 fed brine shrimp for 7 days	14 days and feeding for 7 days	1,2	- 4 days
XVI_{3a}	3	Group XVI_2 fed brine shrimp for 7 days	14 days plus feeding for 7 days	1,2	- 3 days
XVI_{3b}	3	Group XVI_2 starved for another 7 days	21 days	1,2	- 1 day

[a] Experiment 1, 2 and 3 were started on April 30, May 14, and May 23, respectively.

[b] Concentration No. 1 was the highest one followed by 1:1 dilutions producing nominal concentrations of 1/2, 1/4, 1/8 and 1/16 of this concentration. Concentration No. 6 (not presented above) was the control.

[c] Started on May 1.

In order to reveal the ecological significance of toxicity data for these groups of fish another group of approximately 200 fathead minnows (group XVI) were trapped on April 28 and 29 in the pond mentioned above and brought to the laboratory on April 30. After short-term temperature acclimation (25 ± 1°C - 7 hrs) they were put into one of the pretreatment tanks for 24 hrs prior to exposure to endrin.

Fish from groups I through XVI were taken at two or more occasions (as indicated by subscripts, Tables 1 and 2) for toxicity tests with endrin. A summary of the pretreatments for the various groups of fish used in toxicity tests are presented in Tables 1 and 2.

EXPERIMENTAL DIETS

In order to study the influence of lipid content and composition, six groups of fish (I-VI) were fed diets with increasing amounts of lipids. For this purpose six diets were prepared based on the recommendations given for reference research diets (National Research Council, 1973, 1977; Braun and Schoettger, 1975). All of the experimental diets contained the same percentage (dry weight basis) of proteins (lactic casein, and gelatin), digestible carbohydrates (dextrin), vitamins (vitamin mixture 28-997), and minerals (mineral mixture 28-998). The only ingredients and percentages that were varied were the lipid source (corn oil and/or salmon oil), which was compensated for by the nondigestible binder (α-cellulose). All ingredients, except for the salmon oil (relatively free from pesticide residues), which was kindly provided as a gift from Dow Chemical Co., were prepared by Zeigler Brothers, Gardners, Pennsylvania. A description of the artificial diets prepared, I through VI, is presented in Table 3.

The artificial diets, I through VI, were prepared by mixing appropriate amounts of all dry ingredients including the lipid sources (corn and/or salmon oil (Table 3). After careful mixing, the dry powdered diets were stored in sealed plastic boxes at 12°C. Each week (Monday) the daily rations for the whole week, 6 x 7 rations consisting of 10.0 g dry diet mixed with 20 ml water in an aluminum weighing cup, were prepared and stored in 12°C.

In addition, two groups of fish, VII and VIII, were fed frozen brine shrimp (San Francisco Bay Brand, Newark, California 94560; Stock #65006). Group VII was fed essentially *ad lib*, while group VIII, was fed a much lower ration level (see Section 6).

AQUARIA SYSTEM FOR REARING OF FISH

During the pretreatment period, including feeding with diets I through VIII and starvation for periods ranging from 1 to 95 days, all groups of fish were held in a flow through test system previously described by Benoit *et al*. (1976). Briefly described, it consisted of six duplicate upper glass tanks (55 x 28 x 15 cm; L x W x H) with a water volume of 20 l and six duplicate lower stainless steel tanks (91 x 33 x 30 cm; L x W x H) with a

TABLE 3. COMPOSITION OF ARTIFICIAL DIETS (I-VI)

Ingredient	Amount of ingredients (g) in respective diet					
	I	II	III	IV	V	VI
Casein	280	280	280	280	280	280
Gelatin	120	120	120	120	120	120
Dextrin	280	280	280	280	280	280
Vitamin mix.	10	10	10	10	10	10
Mineral mix.	40	40	40	40	40	40
Sum of above	730	730	730	730	730	730
Corn oil	-	100	-	50	75	100
Salmon oil	-	-	100	50	75	100
Sum of above	730	830	830	830	880	920
α cellulose	270	170	170	170	120	70
Sum of above	1000	1000	1000	1000	1000	1000
Added water	2000	2000	2000	2000	2000	2000
Moisture (%)	66.7	66.7	66.7	66.7	66.7	66.7

water volume of 90 l. The water was supplied by a 2 l proportional diluter (Mount and Brungs, 1967) and flow-splitting cells (Benoit and Puglisi, 1973) which provided a total water flow of 1 l/min to each set of duplicate upper and lower tanks. Water replacement times (90%) for each tank were approximately 4.5 hrs (Benoit et al., 1976). Water temperatures in the upper tanks were approximately 25 ± 1°C, while the lower tanks were 26 ± 1°C.

AQUARIA AND TOXICANT DELIVERY SYSTEM FOR TOXICITY TESTS

A system identical to that used for rearing the fish was utilized during the toxicity tests (Holcombe et al., 1976). Data for flow rates and test temperatures are included in Appendix A. A schematic figure of the exposure system is presented in Appendix F.

The toxicant, endrin, was delivered into the exposure system by a saturator (Veith and Comstock, 1975) modified slightly by Jarvinen and Tyo (1978). The column of the saturator was made up by coating 5.0 g of endrin (Analytical Reference Standard; assay: 96.1%, std. no. RS: E-71172, Velsicol Chemical Corp.) dissolved in acetone (P.A.) on 100 g of acetone-washed sand. The delivery of toxicant from the saturator holding tank containing 14 l was achieved by a "dipping bird" (described by Lemke et al., 1979). Thirty-five milliliters of water saturated with endrin was mixed in the diluter on each cycle. Endrin was introduced into the exposure system 20 days prior to exposing the animals in order to equilibrate the system. The concentration of endrin in the saturator was monitored daily by gas chromatography (APHA et al., 1975), Monday through Friday. Accuracy for total endrin in water was checked by the method of known additions by adding endrin to control water and determining the percentage recovery. The mean recovery and 95% confidence interval for the entire exposure period was 89 ± 5%. Precision was determined by analyzing duplicate samples from a different exposure tank with each sampling period. This was repeated 8 times (over period May 1-30) and the results were within 6% of each other. A complete listing of all measured endrin concentrations (from May 1-30) are presented in Appendix C, while endrin concentrations for specific experimental groups are presented in Tables 9, 10, and 11.

WATER CHARACTERISTICS

All fish used in the present study were raised and tested in Lake Superior water. The only exception was the minnows which were trapped in the pond prior to experimental use. Water quality in this pond is, however, considered to be close to Lake Superior water as the pond is located close to the lake and supplied with water from the same drainage area.

Before entering the test system all Lake Superior water was sand filtered, sterilized by ultraviolet light and brought to the desired temperature (25 ± 1°C) in the head-box reservoirs (one for each experimental system).

Weekly water quality data on alkalinity, hardness and pH for the study period are presented in Appendix B. Ranges for pH, alkalinity and hardness were 7.2 to 7.5, 39.5 to 44 mg/l as $CaCO_3$, and 43.5 to 49.5 mg/l as $CaCO_3$, respectively. On one occasion, May 17 and 18, all of the upper exposure tanks were sampled for measurements of dissolved oxygen, pH, alkalinity, acidity and total hardness (data presented in Appendix B).

TREATMENT OF EFFLUENT

All test water leaving the exposure system was put through an activated carbon filter designed to remove >90% of the endrin prior to going to the city sewer system. During the experimental period the percent removal ranged from 82-94%.

SECTION 5

EXPERIMENTAL PROCEDURES

GENERAL PROCEDURES

To enable quantification of minor differences in toxicity to the experimental groups of fish, uniform conditions were created during pretreatment of the test fish prior to testing as well as during the toxicity tests. This was achieved in the following way.

a. Pretreatment of fish

1. Use of an exposure system (without addition of toxicant) which was previously designed to produce uniform conditions in terms of water quality flow, and temperature as well as the physical environment (uniform tanks) and light (diffuse light at 16 hrs/day).

2. Establishment of uniform and realistic feeding routines and exposure system maintenance - siphoning of tanks (daily), cleaning tank sides and bottoms (weekly).

b. Toxicity Testing

1. In addition to the items mentioned above for pretreatment the uniformity of the exposure conditions were preconditioned by careful adjustments of diluter function, especially flow rates to duplicate tanks, and adjustment of temperature in the headbox.

2. Uniformity in exposure conditions was monitored and controlled by measurements of variations between and within duplicate tanks.

3. By separating the groups of fish to be compared by stainless steel screens, as all diet groups were exposed in the same, continuously renewed solution of toxicant (five concentrations plus one control).

4. Possible timewise differences in water quality, temperature and toxicant concentrations as well as timewise differences in pretreatment were eliminated by starting experimental exposures of groups to be compared on the same day (Experiments 1-4). In the present study the two main timewise differences were the adjustment of flow-rates in the upper tanks on May 7 and variations in the concentrations of endrin in the saturator. For further information on these matters see Appendix A and C.

FEEDING ROUTINES

As mentioned previously portions of the food (Diet I-VI) were prepared weekly by weighing out 10.0 g premade powdered dry food and mixing with 20 ml of water. For diets VII and VIII consisting of frozen brine shrimp (Artemia salina) sufficient amounts for 1 week were thawed once weekly, weighed into the same kind of aluminum pans as the other diets. For this purpose, 40.0 g (wet weight, which corresponds to 4.2 g dry weight) was weighed into each pan and stored in a freezer until used that week.

As the fathead minnow does not have a differentiated stomach for the storage of food, a sufficient time for feeding each day must be allowed in order to to obtain optimal growth. It was shown at this laboratory (unpublished observations) as well as by other investigators (Smith et al., 1978) that the growth rate is positively correlated to the number (1-4 times) of feedings per day. In order to create a feeding situation as close to ad lib feeding as possible, the following routine was followed during the entire pretreatment period (February 9 through May 1, 14, and 22, Exp. 1-3). Food previously frozen in aluminum weighing pans, was placed in the same corner of the exposure tank each day (Monday through Friday) between 3 and 4 p.m. On Saturday and Sunday food was placed in the tanks between 9 and 10 a.m. Food was removed between 8 and 8:30 a.m. the following day (all days), taking care to siphon up all spilled food on the bottom. This was usually easy to do, except for the frozen brine shrimp (group VII) in which case residual brine shrimp were siphoned up in about 2 l of water in a plastic bucket. After settling for 15 min the food was concentrated into less than 200 ml and put into new aluminum weighing pans. The residual uneaten food was taken to dryness overnight in an oven at 105°C. By weighing dryed samples of the prepared diets (dry weights 9.2, 9.2, 9.3, 9.3, 9.3, and 9.4 g for I-VI) and the residual uneaten diets, the food consumption for each group of fish was determined as the difference (dry weight basis).

After removal of food from the tanks, tank bottoms were siphoned free of fecal material and the number of dead fish were recorded. Once a week (Monday) all tanks were cleaned by scrubbing and additional siphoning. No visual differences in turbidity of the water between the various tanks was observed.

On February 22 all groups (I-VII) of fish in the upper pretreatment tanks were counted and total body weights determined, after which they were transferred to corresponding lower tanks in the pretreatment system. On May 1, before the start of Experiment 1 (first toxicity test), all groups of fish (I-VII) were counted and total body weights determined.

During the toxicity tests and one day prior to starting tests no food was given. For the starvation groups, no food was added after transfer to the pretreatment system.

During endrin exposure, all tanks were inspected twice daily Monday through Friday and once on Saturday and Sunday. Dead fish were removed and wet weights and body water content (drying at 105°C overnight) were determined on the starvation groups (Appendix E). Data recording on food

consumption and mortality for fish groups I through VII are presented in Appendix D.

TOXICITY TESTING ROUTINES

Randomization of experimental fish for toxicity tests was performed in the following three steps.

1. After transfer of all fish in a group to a 10 l bucket (aerated when necessary), appropriate numbers of fish (8 to 10) for all groups, except group IX where 4 to 5 fish per concentration only were available due to previous starvation mortality, were randomized into 6 (Exp. 1), 3 (Exp. 2), or 2 (Exp. 3) plastic containers (approximately 3 l water).

2. Transfer of fish in each plastic container to various concentrations was randomized.

3. Position of screened compartments was prerandomized (positions presented in Appendix F).

Transfer to exposure compartments was performed with a small dip net. The start of the exposure was recorded for each group of fish (I-XVI). The time required to place fish into the toxicant solutions was less than 3 hrs for groups I through VIII and less than 1 hr for groups IX through XVI in Experiment 1 (started April 30). After a 24 hr acclimation to 25°C, the exposure of groups XV and XVI were initiated on May 1.

Mortality rate and body weight for all groups of fish were recorded in order to reveal possible relationships between body weight and sensitivity to endrin. These measurements were performed more frequently during the first 24 hrs (8 a.m., 1 p.m., 4 p.m., and 11 p.m.) and twice daily thereafter. Dry weights and body water content were also determined on all dead fish as well as survivors at termination in those fish starved prior to exposure (IX-XVI). Both live and dead fish from groups I-VIII were frozen for future residue analysis. Fish (groups I-VIII) from concentrations 4, 5, and 6 (controls) were also sampled (4 to 5 from each group) after 14 and 29 days of exposure to allow the determination of a bioconcentration factor. Results of these residue analyses will be presented in a separate report.

STATISTICAL TREATMENT OF DATA

Median Survival Times

Median survival time (MST) was selected as the endpoint in these endrin toxicity studies. From these data LC50's can be estimated for any period of time within the range where 50% mortality occurred, and, although more uncertain, to periods of time outside this range. For calculation of MST's and 95% confidence limits a computerized procedure (Hamilton et al., 1977) for the Trimmed Spearman-Karber Method of calculating LC50's was used by substituting dose (concentrations) for survival time. The survival time for

each fish was considered to be closest to the time between when mortality was observed and the preceding observation, i.e., (time when the fish was removed and last time observed alive) divided by 2.

SECTION 6

RESULTS

The results of this study are divided into the following subject areas:

1. Dietary effects on the fathead minnow.
2. Starvation and its effects on the fathead minnow.
3. Dietary influence on endrin toxicity
4. Influence of starvation on endrin toxicity
5. Correlations between body water content and susceptibility to endrin

DIETARY EFFECTS ON THE FATHEAD MINNOW (prior to endrin exposure)

During the period lasting from February 9 to May 20 (100 days) mortality and food consumption were measured daily for diet groups I through VII.

In addition, total body weight and total number of fish were determined on January 22 and 23 (during randomization of fish groups for diet study), on February 21 (during transfer from upper to lower tanks) and on April 30 (during start of Exp. 1). Total numbers of fish in each tank (I-VI) were also determined on May 24 (after initiation of the last exposure to endrin, Exp. 3) and on June 12 (after feeding these groups of fish with frozen brine shrimp in order to look for possible recovery from visual abnormalities like lordosis and hemorrhagic kidney regions).

Mortality

Daily recordings of mortalities in the tanks (I-VII) are presented in Appendix D. Cummulative mortality for 100 days (February 9 through May 20) is presented in Table 4. It was found that the brine shrimp diet produced the least mortality.

After 100 days, the cummulative mortalities were similar among the groups fed the artificial reference research diets (I-VI). No influence of either total lipid content (0 to 20%) or lipid type (corn oil in Diet II and salmon oil in Diet III) was observed. The overall tendency, as seen for the sum of the groups (I-VII), was that mortalities occurred at a very constant rate throughout the entire pretreatment period. Although not actually

TABLE 4. CUMMULATIVE MORTALITY OF FATHEAD MINNOWS FED SEVEN DIFFERENT DIETS FOR 100 DAYS

Days of feeding	Diet no. I	II	III	IV	V	VI	VII	(I-VII)
0	0*(0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
50	10.5 (23)	8.6 (19)	10.0 (22)	7.3 (16)	13.2 (29)	3.6 (8)	0.5 (1)	4.3 (118)
100	23.6 (52)	28.2 (62)	29.1 (64)	21.8 (48)	24.5 (54)	20.5 (45)	0.9 (2)	21.2 (327)

* Cummulative mortality expressed as precentage of initial number, which was 220 fish for each dietary treatment. Number in parenthesis is cummulative mortality.

quantified, size and body weight of dead compared to live fish seemed to follow the same pattern, i.e., dead fish were neither smaller nor larger than live fish. Infectious disease as a course of death was suspected on a few occasions, but no general trends were observed. Therefore, the reasons for the higher mortalities among fathead minnows fed the artificial diets (I-VI) compared to the brine shrimp diet (VII) is so far not understood; although, one would expect dietary deficiencies to play a major role. One might speculate that the reason for this difference does not originate in the lipid soluble components, as no real difference in mortality was observed between group I, with no added lipid source, and the other groups (II-VI) fed diets containing corn oil, high in Ω6 fatty acids, salmon oil, high in Ω3 fatty acids, or a 1:1 mixture of them in increasing amounts.

Growth and Food Consumption

The effects of diet on growth (Table 5 and Fig. 1) were in agreement with those on mortality, showing that brine shrimp produce better growth and survival than the artifical diets.

Experimental data on food consumption (Appendix D) have not been fully evaluated. An interpretation of these data must consider actual number of fish as well as their body weight. This can be estimated from the data on total body weight, mortality and total number of fish in groups I-VII. Data for caloric content, which varies among the diets, and digestibility are also of importance in such an evaluation. However, the need for a more detailed interpretation is not obvious because of the poor growth and survival among fish fed the artifical diets.

Other Effects of Diet

In addition to the increased mortality and reduced body weight gain, observed in fathead minnows fed artifical diets (group I-VII), live fish showed visual symptoms of dietary deficiencies. These were not observed in brine shrimp-fed fish (group VII and VIII). Symptoms of deficiency which were observed in some fish fed artifical diets included swollen bellies, lordosis, hemorrhages (especially of the kidney region) and erratic swimming. In severe cases, these fish eventually died. Some fish were, however, able to survive with these symptoms for several weeks.

After the last exposures to endrin were started, the total number of remaining unexposed fish in group I-VI and the number of visually abnormal fish with lordosis and/or hemorrhagic kidney regions were counted. These fish were then fed frozen brine shrimp once daily, 5 days a week, for 3 weeks, after which they were differentially counted again. The results of this alteration in diet is shown in Table 6. The good recovery of fish in group IV (50%) compared to that for the other groups (0-15%), and probably the recoveries in group III (15%) as well, was due to a high incidence of hemorrhagic kidney lesions observed in these groups. The hemorrhagic kidney lesions were not observed prior to the last 2 weeks of the feeding period, when the number of fish affected in this way seemed to increase. At that time fish with hemorrhagic kidney lesions were also observed in group III and V. Only one dead fish with this symptom was observed.

TABLE 5. MEAN BODY WEIGHTS AND MEAN DAILY BODY WEIGHT GAIN FOR FATHEAD MINNOWS FED SEVEN DIFFERENT DIETS AD LIB

Date	Diet Group I	II	III	IV	V	VI	VII
01-23-79	.091 (220)[a]	.098 (220)	.093 (220)	.095 (220)	.091 (220)	.095 (220)	.092 (220)
	Feeding with frozen brine shrimp						
02-09-79	Feeding with experimental diets initiated						
02-21-79	.239 (218)	.251 (216)	.255 (208)	.269 (217)	.210 (218)	.230 (222)	.340 (213)
Mean daily body weight gain[b]	.00510	.00528	.00559	.00600	.00410	.00466	.00855
04-30-79	.514 (147)	.688 (170)	.708 (160)	.751 (185)	.538 (154)	.536 (168)	1.175 (211)
Mean daily body weight gain[c]	.00404	.00643	.00666	.00709	.00482	.00450	.01228

[a] Mean body weight (g) and number of live fish in parenthesis.

[b] Mean body weight (g) at 02-21-79 minus that at 01-23-79 through number of days in between (29).

[c] Mean body weight (g) at 04-30-79 minus that at 02-21-79 through number of days in between (68).

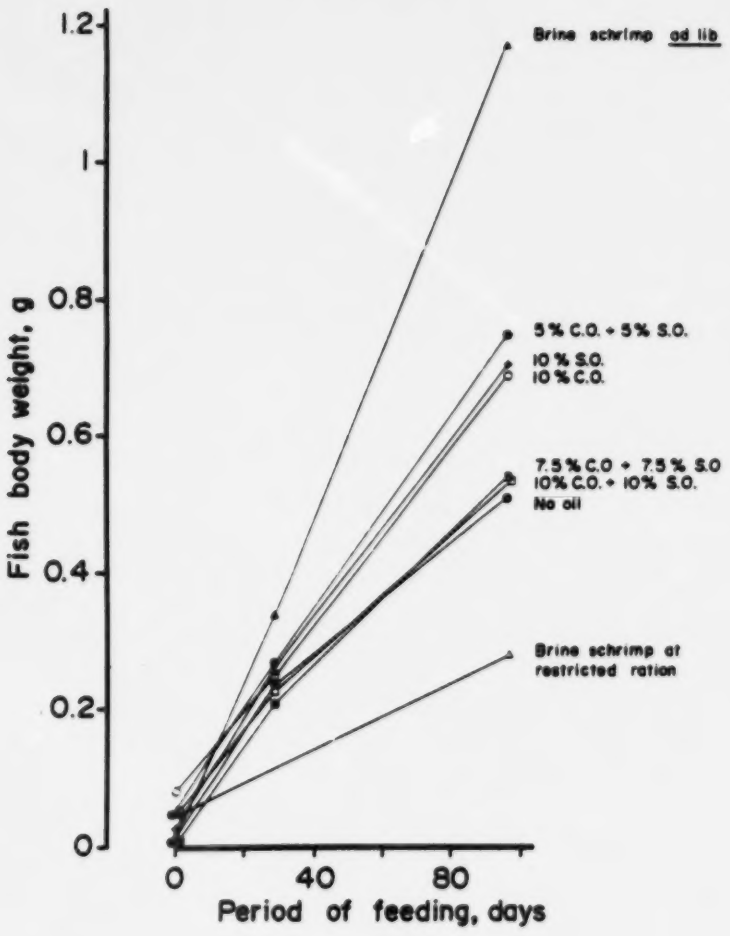

Figure 1. Growth in fathead minnows fed 7 different diets. Percentages shown are for the dry diets. C.O. (Corn Oil) and S.O. (Salmon Oil). Composition of experimental diets is presented in Table 3.

TABLE 6. RECOVERY OF ABNORMAL FATHEAD MINNOWS WHEN FED FROZEN BRINE SHRIMP FOR THREE WEEKS

Diet group no.	I	II	III	IV	V	VI
Initial total no. of fish	5	48	36	66	43	50
Initial no. of abnormal[a] fish	1	2	13	28	13	10
Mortalities[b]	0	0	1	3	2	2
Final total no. of fish	5	48	35	63	41	46
Discrepancy	0	0	0	0	0	-2
Final no. of abnormal fish	1	2	10	10	10	6
Recovery	0/1	0/2	2/13	15/28	1/43	0/10
Recovery (%)	0	0	15	50	2	0[c]

[a] Visually abnormal fish. This number includes fish with lordosis and/or hemmorrages.

[b] Occurred within the first week of feeding with brine shrimp.

[c] It is assumed that two of the abnormal fish were eaten by the remaining fish.

The hemorrhagic kidney lesions could have been caused by some product produced during storage of the dry diets, as no dose-dependent relationship with any of the ingredients existed. The only possible connection between a dietary ingredient and the hemorrhagic kidney lesions is the salmon oil. One possible explanation is, that some of its ingredients, e.g. the polyunsaturated fatty acids, had deteriorated and thus produced nephrotoxic components.

In contrast to the recovery of the hemorrhagic kidney lesions, no recovery from lordosis was observed. Lordosis occurred in all groups fed the artifical diets (I-VI), but not in group VII and only to a minor extent in group VIII, which were both fed frozen brine shrimp.

STARVATION AND ITS EFFECTS ON THE FATHEAD MINNOW (prior to endrin exposure)

When starved at 25°C adult fathead minnows survived for about 40 days with less than 10% mortality (Fig. 2). After 40 days, mortality due to starvation increased gradually and 50% mortality was reached at about 100 days. The survival time for adult fathead minnows, when starved at 25°C, ranged from 12 days (1 fish in group X) to >126 days (1 surviving control in group IX). For juvenile fish (33 days post-hatch; average body weight 0.095 g) starvation resulted in 50% mortality after 17.5 days (23.0-24.5°C) as seen in Fig. 3.

Independent of survival time, fish killed by starvation had a body water content (post-mortem determination) of 86.8±2.2% (Mean ± S.D. for fish in group IX which were killed by starvation over a 32-95 day period) (see Appendix E). This can be compared with the water content of surviving control fish from group IX-XVI presented in Table 7. Surviving fish were killed by immersion in ice-water for 1-2 min prior to determination of total body weight and water content. From the data presented in Table 7 it is evident that fish killed by starvation had a higher water content than surviving fish. Furthermore, based on the total body water content of surviving fish starved for periods between 30 and 126 days and that of the pond-fish trapped just prior to testing on April 30 (Table 7), it can be concluded that the pond-fish after 30 days starvation at 25°C, had a nutritional status similar to laboratory reared fish starved for about 67 to 98 days (80 days for simplicity). A subtraction of the 30 days of starvation, when these fish served as controls, then suggest that these fish (group XVI) had a nutritional status similar to fish starved for 50 days (80 minus 30) at 25°C.

DIETARY INFLUENCE ON ENDRIN TOXICITY

As mentioned above diet *per se* was shown to affect the fathead minnow in terms of growth, survival and gross pathology. Only healthy looking fish were taken for bioassays with endrin. As shown in Table 8 their mean body weights (determined post-mortem) differed slightly among the various groups. However, there was no detectable relationship between body weight of fathead minnows and susceptibility to acute endrin toxicity. Furthermore, it was

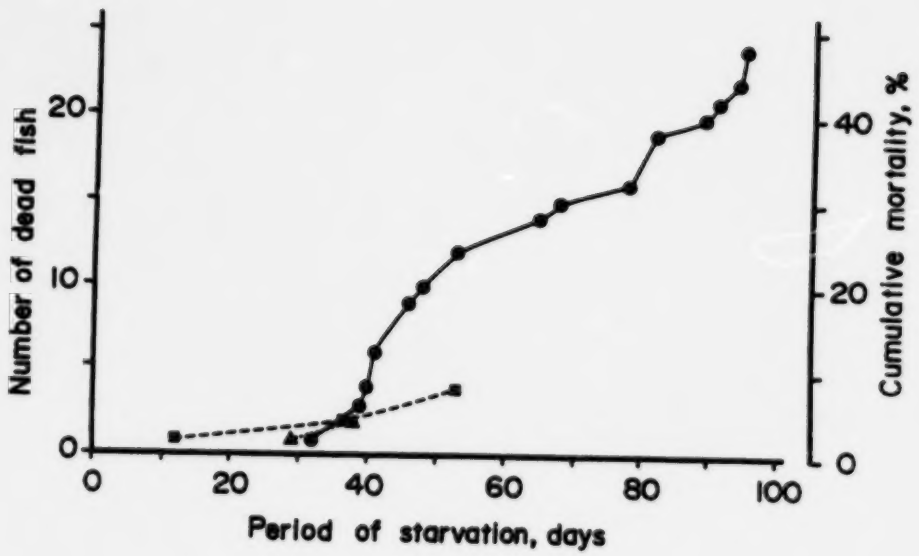

Figure 2. Mortality of adult fathead minnows starved at 25.0° C. The symbols in the figure are for group IX (●-----●), X (-----), and XI (▲-----▲).

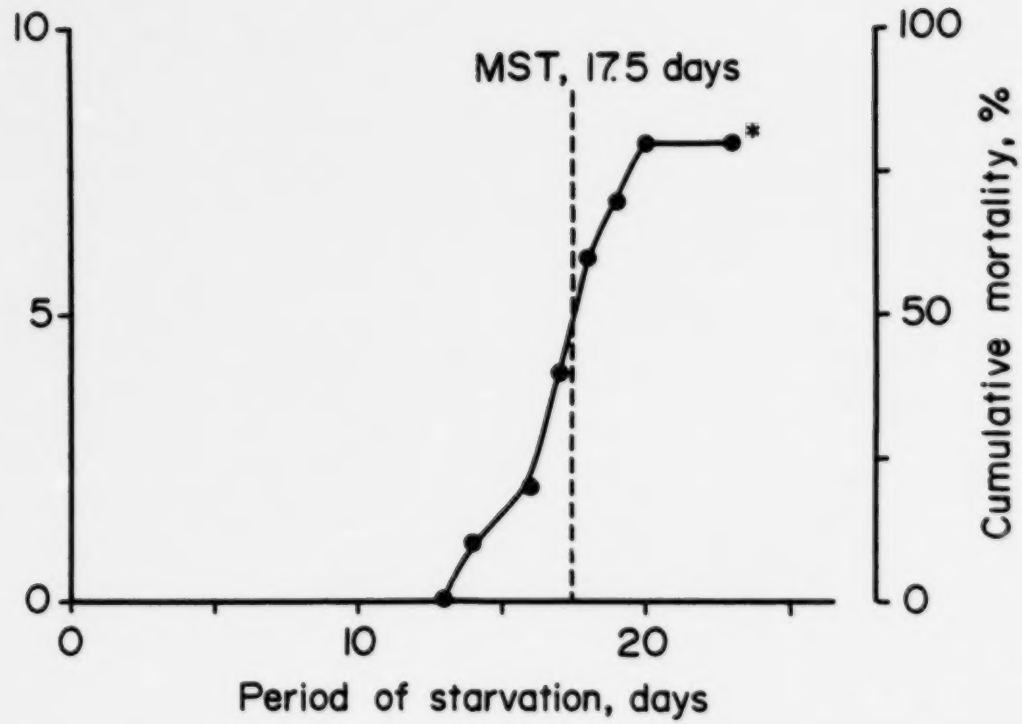

Figure 3. Mortality of juvenile fathead minnows starved at 23.0-24.5° C. *The experiment was terminated after 23 days. Age and weight at day 0 was 33 days and .095 g, respectively.

TABLE 7. WATER CONTENT (%, w.w/w.w.) OF SURVIVING AND STARVATION-KILLED FATHEAD MINNOWS STARVED FOR VARIOUS PERIODS AT 25°C

Group of fish[a]	IX	X	XI	XII	XIII	XIV	XV	XVI
Period of starvation (days)	126	98	67	50	30	92	30	30
Water content of surviving fish (%)[b]	82.6 (1)	82.9 (5) ± 2.8	81.9 (7) ± 3.3	79.6 (7) ± 4.7	74.0 (10) ± 3.1	83.4 (7) ± 2.7	78.9 (10) ± 3.1	82.1 (7) ± 1.9
Water content of dead fish (%)[b]	88.8 (2) ± 0.8	87.0 (1)	- (0)	- (0)	- (0)	- (0)	- (0)	87.6 (2) ± 2.5

[a] Group IX-XIII originated from the laboratory stock, and were fed frozen brine shrimp at 25°C prior to starvation. Group XIV and XV originated from a pond and were fed trout starter at 5-10°C in the laboratory during the winter for 5 and 7 months respectively prior to starvation. Group XVI were fathead minnows trapped in the same pond just prior to starvation.

[b] Results are expressed in (%) (= g water/100 g on a wet weight basis) and presented as Mean ± S.D. with number of fish (n) in parenthesis.

TABLE 8. BODY WEIGHTS* OF FATHEAD MINNOWS PRE-FED WITH EIGHT DIETS AND USED IN TOXICITY TESTS WITH ENDRIN

Group of fish	I	II	III	IV	V	VI	VII	VIII
				Experiment 1. (April 30-May 7)				
Concentration 1	.676 (10) ± .321	.797 (9) ± .244	.739 (10) ± .404	.764 (10) ± .274	.558 (10) ± .244	.694 (10) ± .287	1.502 (10) ± .737	.623 (10) ± .256
Concentration 2	.570 (10) ± .140	.795 (10) ± .311	.610 (10) ± .135	.587 (10) ± .242	.471 (10) ± .147	.428 (10) ± .103	.928 (9) ± .282	.534 (10) ± .322
Concentration 3	.588 (10) ± .137	.596 (10) ± .159	.759 (10) ± .146	.700 (10) ± .183	.596 (10) ± .263	.466 (10) ± .130	1.273 (10) ± .374	.520 (10) ± .152
				Experiment 2. (May 14-May 30)				
Concentration 1	.540 (10) ± .222	.816 (10) ± .314	.869 (10) ± .425	.982 (10) ± .383	.887 (10) ± .245	.544 (10) ± .179	1.929 (10) ± .672	.315 (10) ± .122
Concentration 2	.614 (10) ± .281	.840 (10) ± .141	.784 (10) ± .427	1.187 (10) ± .340	.697 (9) ± .236	.616 (10) ± .209	1.607 (10) ± .485	.330 (10) ± .181
Concentration 3	.550 (9) ± .328	.746 (8) ± .284	.720 (11) ± .218	.859 (9) ± .175	.539 (11) ± .188	.465 (8) ± .156	1.670 (9) ± .681	.350 (9) ± .223

* Body weights (g) expressed as Mean ± S.D. with number of fish (n) in parenthsis. Body weights determined on fish killed either by endrin during tests or by immersion in ice-water at termination.

not possible to visually determine the dietary treatment of individual fish, as variations in size and shape was as great within the groups as between them. Consequently, the observed differences in susceptibility to endrin among the dietary groups originated from invisible, internal, biochemical and physiological differences. These differences were in turn created by the diets fed to the fish.

Before examining the differences or similarities in susceptibility to endrin among the groups of fish (I-VIII), it is important to know how exact and reproducible the method for determination of toxicity is. This can be done by examining the results obtained for group VIII, the group fed a restricted ration of frozen brine shrimp.

From the results presented in Table 9 and Fig. 4, it can be seen that the relatively small discrepancies obtained can be attributed to the slight differences in exposure starting times. When different groups of fish from the same period (May 8-11) are exposed to the same endrin concentration, but in different exposure chambers, then the differences in MST are small (<2.2% maximal difference for the mean values).

From the data presented in Table 10, it is evident that fish fed the diet with no added lipids (group I) was most susceptible, and that fish fed frozen brine shrimp ad lib (group VII) was most resistant. Looking at Figure 5 and comparing the 96-hr LC50s of .485 and 1.15 µg/l interpolated from it, the maximal variation in endrin toxicity due to diet becomes 2.4 times (1.15/.485). Within the periods of exposure examined (<384 hrs or 16 days), no incipient lethal threshold was established.

Comparing the susceptibility to endrin for fish fed various percentages or composition of lipid in their diet, the only consistent difference was found between fish fed no added lipid (group I) and the other groups (II-VI). Consequently, a variation in dietary lipid percentage between 10% (groups II, III and IV) and 20% on a dry weight basis (group VI) seems to be of minor importance in terms of susceptibility to endrin. Furthermore, there was no difference in susceptibility to endrin among fish fed a diet containing corn oil, which is high in Ω6 fatty acids (group II), and fish fed a diet containing salmon oil, which is high in Ω3 fatty acids (group III).

From the data presented in Table 10 and Figure 4, the influence of feeding a restricted ration of brine shrimp (group VIII) and a high ration level, essentially ad lib (group VII) can be examined. On April 30, after 80 days of feeding, group VIII had 733 fish with a total body weight of 204.5 g (average body weight .279 g). They were fed a daily ration of 40.0 g frozen brine shrimp (4.8 g dry weight) for the entire period (80 days) prior to April 30. Based on an average initial body weight of .095 g/fish (determined from groups I-VII, Table 4) and an assumed initial number of 745 (based on the final number, 733, and mortality data for group VII, Table 4), an average total body weight during the feeding period for group VIII can be calculated as (204.5-745 x .095)/2 = 66.86 g. The average daily ration fed to group VIII then becomes 40.0 x 100/66.86 = 59.8 g/100 g body weight on a wet weight/wet weight basis or 7.18 g/100 g body weight on a dry weight/wet

TABLE 9. TOXICITY OF ENDRIN TO FATHEAD MINNOWS FED A LOW RATION OF BRINE SHRIMP PRIOR TO TOXICITY TESTS, WHEN TESTED AT VARIOUS PERIODS AND IN DIFFERENT EXPOSURE CHAMBERS

Period of exposure	Conc. 1 (µg/l)	MST (hrs)	Conc. 2 (µg/l)	MST (hrs)	Conc. 3 (µg/l)	MST (hrs)
April 30-May 7	1.37[a]	34.5[a]	0.63	96.6	-	-
	1.34-1.39	30.5-39.1	0.48-0.77	84.9-109.8	-	-
May 14-May 30	1.29	23.8	0.71	71.6	0.39	178[b]
	1.23-1.35	19.4-29.2	0.52-0.90	60.7-84.5	0.30-0.48	-
May 23-May 27	1.61	21.6	0.79	41.1	-	-
	1.55-1.68	17.6-26.6	0.76-0.83	33.8-50.0	-	-
May 8-May 11[c]	1.51	28.1	-	-	-	-
	1.11-1.91	25.0-31.5	-	-	-	-
	"	29.1	-	-	-	-
	"	25.8-32.9	-	-	-	-
	"	28.3	-	-	-	-
	"	26.1-30.6	-	-	-	-
	"	28.8	-	-	-	-
	"	25.9-32.0	-	-	-	-

[a] Results expressed as Mean and 95% confidence limits.

[b] No confidence limit due to only 30% mortality. Median survival time (MST) based on plot of mortality data on log-probit paper.

[c] Exposures performed in 4 adjacent upper chambers, with the same endrin concentration.

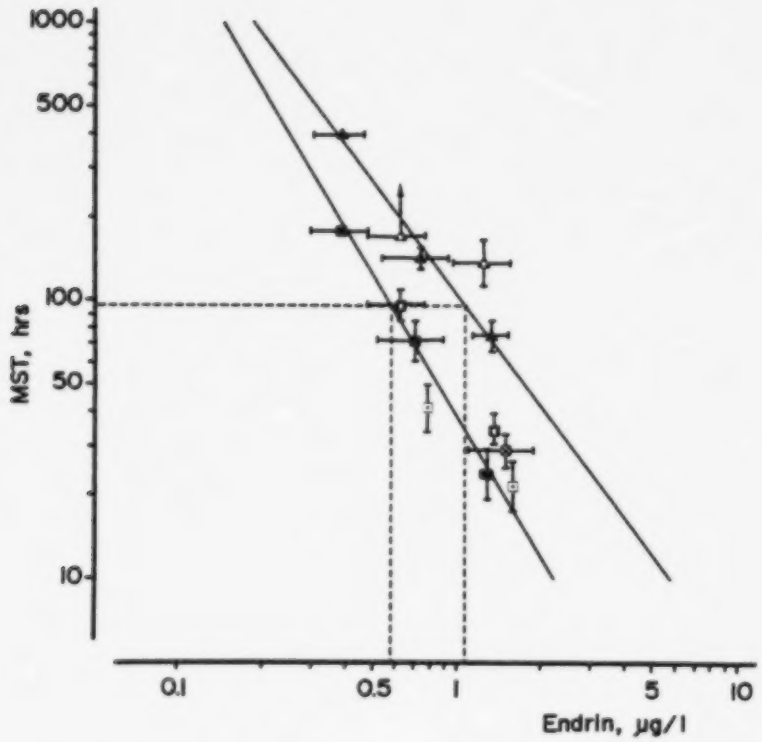

Figure 4. Toxicity of endrin to fathead minnows fed either a high or a low ration of frozen brine shrimp prior to exposure. Results are shown as mean values and 95% confidence limits. Symbols are as follows:

Experiment no.:	1	2	3	4
Diet group VII:	△	▲		
Diet group VIII:	□	■	⊡	⊠

TABLE 10. TOXICITY OF ENDRIN TO FATHEAD MINNOWS FED EIGHT DIETS FOR 80-100 DAYS PRIOR TO TOXICANT EXPOSURE

Diet group	Conc. 1 (ug/l)	MST (hrs)	Conc. 2 (ug/l)	MST (hrs)	Conc. 3 (ug/l)	MST (hrs)
I_1[a]	1.30[b] 1.09-1.51	23.0[b] 17.2-30.9	.63 .48-.77	76.9 40.5-146.0	- -	- -
I_2	1.28 1.22-1.35	19.2 16.5-22.4	.67 .57-.76	55.4 46.4-66.2	.39 .29-.48	138.9 112.4-171.7
I_{3a}	1.68 1.59-1.76	7.0 4.7-10.4	.83 .79-.88	10.3 6.5-16.5	- -	- -
I_{3b}	1.68 1.59-1.76	6.3 4.2-9.4	.80 .75-.86	46.0 36.0-58.6	- -	- -
II_1	1.26 .97-1.56	92.2 78.5-108.2	.63 .48-.77	>170 -	- -	- -
II_2	1.28 1.23-1.34	35.1 26.0-47.4	.74 .54-.93	122.2 101.5-147.3	.39 .30-.48	340 -[c]
III_1	1.30 1.09-1.51	54.7 43.4-69.0	.63 .48-.77	156 -	- -	- -
III_2	1.28 1.23-1.34	43.6 37.0-51.3	.71 .52-.90	81.6 69.8-95.5	.39 .30-.48	246.5 201.3-301.9
IV_1	1.30 1.09-1.51	55.9 46.0-68.0	.63 .48-.77	>170 -	- -	- -
IV_2	1.43 1.05-1.18	28.9 20.5-40.7	.74 .55-.93	131.5 110.0-157.3	.39 .31-.47	384.6 327.5-451.7
V_1	1.36 1.32-1.40	53.5 43.2-66.2	.63 .48-.77	245 -	- -	- -
V_2	1.34 1.43-1.53	53.8 48.8-59.5	.74 .55-.93	106.5 92.9-122.2	.39 .31-.47	261.3 189.8-359.8
VI_1	1.30 1.09-1.51	74.6 67.8-82.2	.63 .48-.77	- >170	- -	- -
VI_2	1.38 1.09-1.67	45.3 37.4-54.9	.75 .56-.93	130.6 110.5-154.4	.39 .31-.47	309.2 220.3-433.9
VII_1	1.26 .97-1.56	137.0 113.2-165.6	.63 .48-.77	>170 -	- -	- -
VII_2	1.34 1.14-1.53	74.8 66.0-84.2	.74 .54-.93	142.7 130.4-156.1	.39 .31-.47	396 -
$VIII_1$	1.37 1.34-1.39	34.5 30.5-39.1	.63 .48-.77	96.6 84.9-109.8	- -	- -
$VIII_2$	1.29 1.23-1.35	23.8 19.4-29.2	.71 .52-.90	71.6 60.7-84.5	.39 .30-.48	178 -
$VIII_3$	1.61 1.55-1.68	21.6 17.6-26.6	.79 .76-.83	41.1 33.8-50.0	- -	- -

[a] Symbols explained in terms of pretreatment and exposure in Table 1.

[b] Results expressed as Mean and 95% confidence limit.

[c] No confidence limit, due to insufficient mortality (50%).

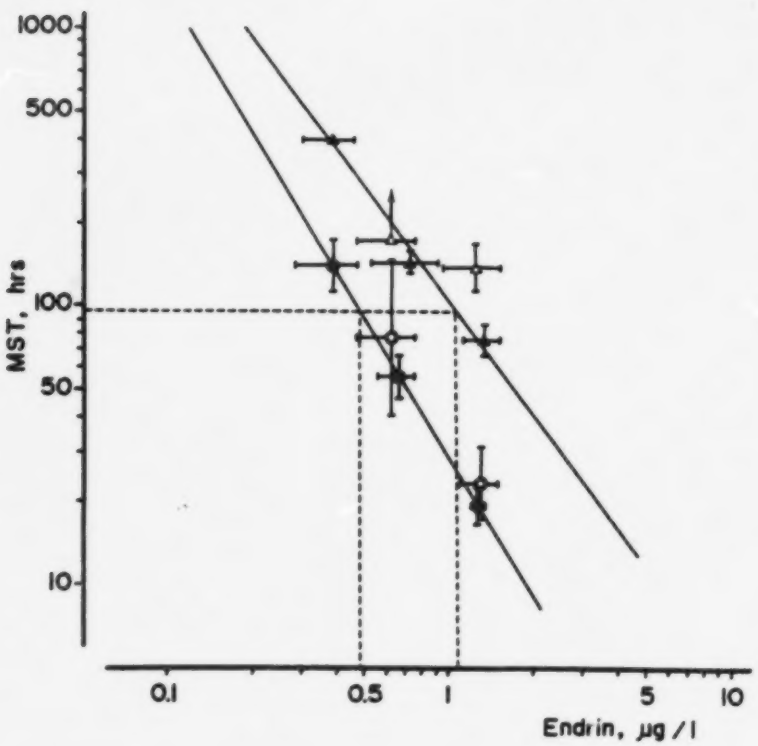

Figure 5. Toxicity of endrin to fathead minnows fed either a low-fat diet or frozen brine shrimp prior to exposure. Results are shown as mean values and 95% confidence limits. Symbols are as follows:

Experiment no.:	1	2
Diet group I:	○	●
Diet group VII:	△	▲

weight basis. Group VII, which was fed essentially ad lib, constituted initially 220 fish with a total body weight of 20.24 g (average body weight 0.092 g). On April 30 there was 211 fish left with a total body weight of 248.0 g (average body weight 1.175 g). The average total body weight for group VII then becomes (248.0-20.24)/2 = 113.9 g. From the daily recordings on food consumption, a mean daily ration of 18.57 g dry weight or 71.45 g wet weight can be determined (for further details see Appendix D). The average daily ration fed to group VII then becomes 62.7 g/100 g body weight on a wet weight/wet weight basis or 7.52 g/100 g fish on a dry weight/wet weight basis. What this means is not that the fish were fed a similar ration (59.8 and 62.7 g/100 g body weight respectively) but just that the food conversion efficiency was similar in both groups. When the daily ration is instead expressed in grams of food per fish independent of body weight, then it can be calculated that fish in group VII were given a 6.15 times higher daily ration than fish in group VIII (71.45/214.5 : 40.0/739 = 6.15). This difference caused apparent differences in susceptibility to endrin as seen in Figure 4, from which 96-hr LC50s of 1.15 and .58 can be interpolated. The difference in susceptibility is 1.98 times. Thus, a low ration of food can create the same order of increase in susceptibility to endrin as a low-fat diet compared to a brine shrimp diet.

INFLUENCE OF STARVATION ON ENDRIN TOXICITY

From the results presented in Table 11 and Figure 6 it is quite clear that prolonged starvation made the fish more susceptible to endrin. Furthermore, it was quite clear that the fish trapped in the pond just prior to toxicity tests (April 30; group XVI), were among the most susceptible groups In fact they were as susceptible to endrin as the fish starved for 95 days prior to toxicity tests (group IX).

From the linear regression line for MST as a function of period of starvation prior to testing (Fig. 6), an equivalent period of starvation (25°C) for the pond-minnows, which had lived in their natural environment during the winter, was determined based on their susceptibility to endrin. These fish behaved as if they were starved at 25°C for 75 days (concentration 1), 95 days (concentration 2) or 96 days (concentration 3). This estimated starvation period was compared to the one independently estimated from total body water content (50 days). The discrepancy between these estimates (50-96 days) becomes easier to understand by looking at Figure 2 and Figure 7. During periods of starvation exceeding 50 days, mortality due to starvation complicates the correlation between water content and period of starvation. Because of this, the increase in water content of surviving fish becomes less as the upper limit is approached. Therefore, an estimate of the prehistory of feeding for fathead minnows with a total body water content greater than about 81% was less precise than below 81%. This might in part explain the discrepancy between the estimate of 50 days based on water content and the estimate of 75-96 days based on susceptibility to endrin. These results showed that fathead minnows at this time of the year (April 30) are starved and that they behaved like starved fish in their response to endrin.

TABLE 11. TOXICITY OF ENDRIN TO ADULT FATHEAD MINNOWS STARVED FOR 1-97 DAYS AT 25°C PRIOR TO TOXICANT EXPOSURE

Group of fish[c]	Conc. 1 (µg/l)	MST (hrs)	Conc. 2 (µg/l)	MST (hrs)	Conc. 3 (µg/l)	MST (hrs)	Conc. 4 (µg/l)	MST (hrs)
IX[a]	1.27[b] 1.36-1.38	7.6[b] 3.0-19.0	.68 .68-.69	9.8 6.6-14.7	.35 .26-.44	61.0 36.3-98.2	.19 .14-.25	91.4 48.5-172.1
X	1.37 1.36-1.38	8.4 5.1-14.1	.65 .54-.75	38.9 22.2-67.6	.35 .26-.44	62.8 40.3-97.4	.19 .14-.25	379.7 31.1-4642.6
XI	1.37 1.34-1.39	15.4 10.7-22.3	.65 .54-.75	59.5 45.4-78.0	.36 .26-.47	176.3 131.8-236.0	.19 .14-.25	409.1 202.3-827.6
XII	1.37 1.36-1.38	16.2 11.1-23.6	.65 .49-.81	97.0 65.0-144.6	.35 .25-.46	220.0 154.8-312.7	.19 .14-.25	>720 -
XIII	1.37 1.34-1.39	26.7 20.2-35.3	.65 .49-.81	65.8 49.6-87.4	.36 .26-.46	228.3 188.9-276.1	.19 .14-.25	501 -
XIV	1.37 1.36-1.38	10.8 7.9-14.7	.68 .67-.69	27.8 20.4-37.8	.35 .26-.44	109.1 83.5-142.4	.19 .14-.25	307 -
XV_1	1.37 1.36-1.37	7.1 4.3-12.0	.64 .53-.95	67.8 55.4-82.9	.37 .26-.47	197.3 144.5-269.4	.20 .14-.25	>720 -
XV_2	1.29 1.23-1.35	23.3 20.4-26.6	.65 .60-.70	46.4 40.0-53.8	- -	- -	- -	- -
XV_3	1.61 1.55-1.68	18.2 13.9-23.8	.79 .76-.83	47.1 39.5-56.1	- -	- -	- -	- -
XVI_1	1.37 1.37-1.37	8.2 5.3-12.5	.68 .67-.69	15.0 10.8-21.0	.32 .25-.39	44.0 33.2-58.3	.20 .14-.25	>720 -
XVI_2	1.28 1.22-1.35	9.2 8.0-10.5	.64 .61-.67	13.8 13.4-18.6	- -	- -	- -	- -
XVI_{3a}	1.61 1.55-1.68	13.4 9.9-18.1	.80 .76-.83	31.6 25.7-38.9	- -	- -	- -	- -
XVI_{3b}	1.63 1.58-1.68	10.7 8.1-14.4	.80 .77-.83	13.0 9.6-17.5	- -	- -	- -	- -

[a] Median survival times (MTS) for Conc. 5 and the control were >720 and 117.6 (45.8-302.2), respectively in Group IX.

[b] Results expressed as Mean and 95% confidence limits.

[c] For information on source of fish and period of starvation, see Table 2.

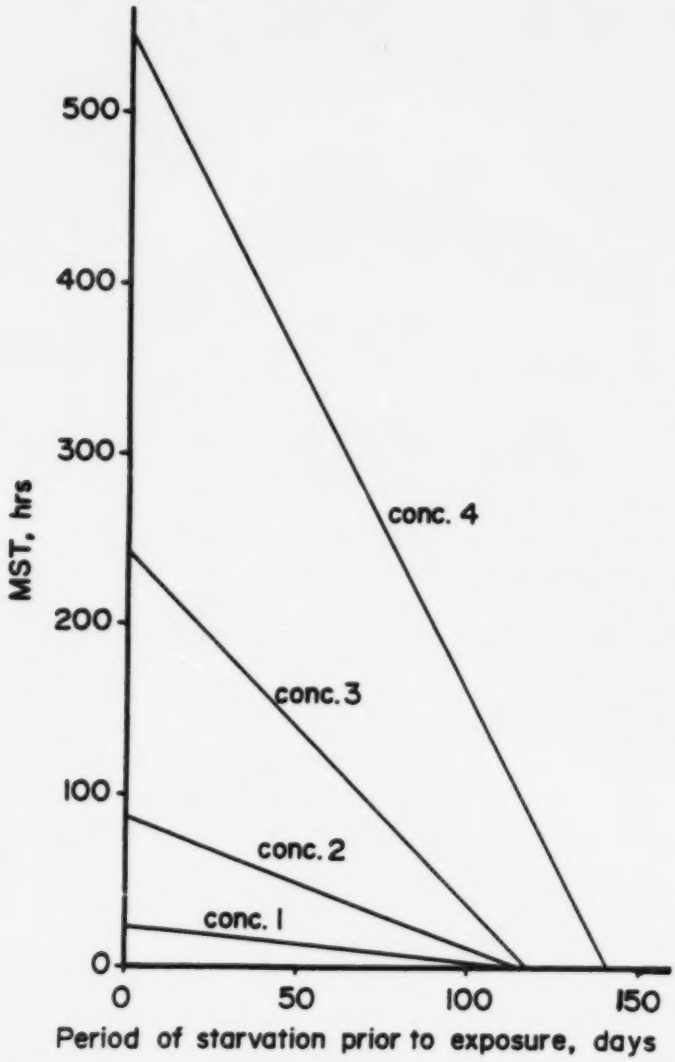

Figure 6. Endrin toxicity to fathead minnows starved for 1 to 97 days prior to exposure.

Data for linear regression analysis (MST's for group IX-XIV) are presented in Table 11. Endrin concentrations (µg/l) are ranges for corresponding mean values. Correlation coefficient (r), slope (a_1), y-intercept (a_o), standard deviation (s) and number of data points were as follows.

Conc. no.	1	2	3	4
µg Endrin/l	1.37	0.65–0.68	0.35–0.36	0.19–0.20
r	-0.930	-0.859	-0.956	-0.899
a_1	-0.188	-0.760	-2.069	-3.839
a_o	23.3	86.4	242.6	543.4
s	2.4	14.4	20.3	60.4
n	6	6	6	6

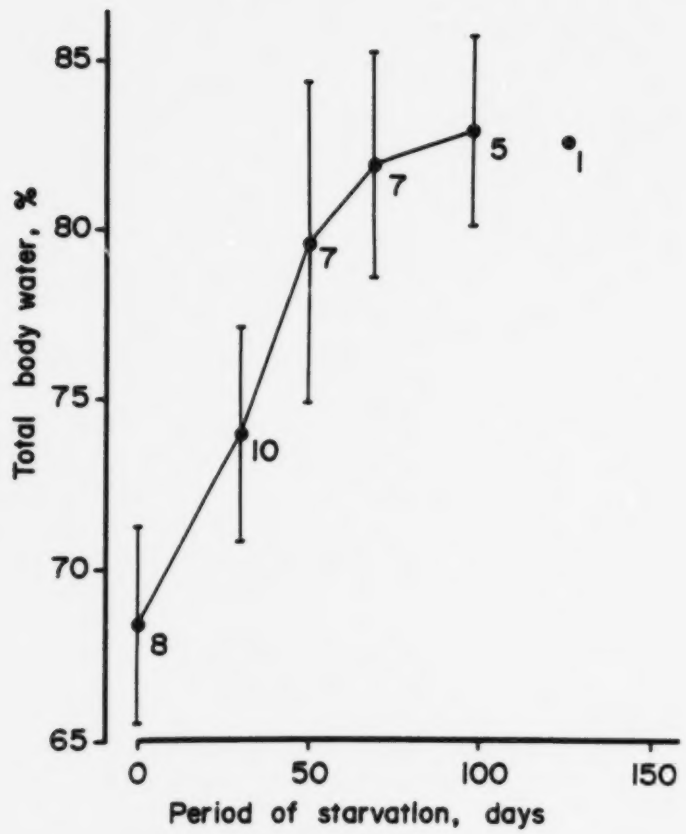

Figure 7. Effect of starvation on total body water in fathead minnows. Each point is a mean of the number of animals listed by each point and ther vertical line through each point is the standard deviation.

When the recently trapped pond-fish (group XVI_1) were starved in the laboratory at 25°C for 14 to 23 days prior to exposure (group XVI_2 and XVI_{3b} respectively) their susceptibility to endrin was not altered much (Table 11). Thus, it seemed that the response to endrin after acclimation from a temperature of about 5°C in the pond to the test temperature of 25°C was at least not influenced much by periods of acclimation between 2 and 23 days prior to exposure to endrin. When fish acclimated and starved for 14 days at 25° C (group XVI_2) were either starved (group XVI_{3b}) or fed with frozen brine shrimp (group XVI_{3a}) essentially ad lib (7.23 g/100 g fish on a dry weight/wet weight basis or 98.2 g/100 g fish on a wet weight/wet weight basis per day during one week) and tested simultaneously, an increased resistance in fed compared to starved pond fish was observed (Table 11). A similar positive response to feeding with frozen brine shrimp for one week prior to testing was also observed in fish previously fed a low fat diet (group I_{3a} and I_{3b} in Table 10).

CORRELATION BETWEEN WATER CONTENT AND SUSCEPTIBILITY TO ENDRIN

In several studies it was shown that the tissue water content of fish was inversely proportional to the fat or lipid content (Love, 1970). Although such a fat-water relationship was not actually shown for the fathead minnow, there is no reason to believe that this relationship should not exist. In the present study the total body water content, expressed as percent on a wet weight basis, was used as a simplified, indirect measurement of the relative total body lipid content. Thus, if a negative correlation between survival time (resistance) and percent water was found, then a positive correlation between survival time and percent lipid was highly probable. Such a correlation would mean that a fat fish can withstand longer periods of exposure or higher concentrations of a lipophilic chemical than a lean fish.

Total body water content for the diet-treated groups (I-VIII) are presented in Table 12. Data for total body water in prestarved groups (IX-XVI) were also determined (Appendix E). Mean values for intoxicated fish in the three highest concentrations of endrin were calculated. In order to see if there was a correlation between total body water and susceptibility to endrin, mean values for total body water content were plotted against median survival times for group I to XVI in the three highest toxicant concentrations, and linear regression analysis was applied to these three sets of data. As seen in Figure 8, a negative correlation existed for all three concentrations. Considering the fat-water relationship (negative correlation) this should support the hypothesis of a protective effect against endrin toxicity by an increased lipid content.

If this relationship is true, then survival time for individual fishes in a certain concentration of endrin should be positively correlated to their respective lipid content. Again considering the fat-water relationship, survival time should then be negatively correlated to water content. The latter was the case (Fig. 9) and represented a negative correlation between total body water content and survival time for individual fishes (group IX-XIII) exposed to the three highest concentrations of endrin.

TABLE 12. TOTAL BODY WATER CONTENT IN FATHEAD MINNOWS FED EIGHT DIETS FOR THREE MONTHS

Diet no.	I	II	III	IV	V	VI	VII	VIII
	78.8* ± 2.8	73.3 ± 2.1	73.5 ± 1.5	70.1 ± 2.3	73.1 ± 2.6	71.6 ± 2.6	68.4 ± 2.9	73.3 ± 2.5

* Mean ± S.D. for total body water (% on w.w. basis). Body water determined on a random sample of 8 fish for each group.

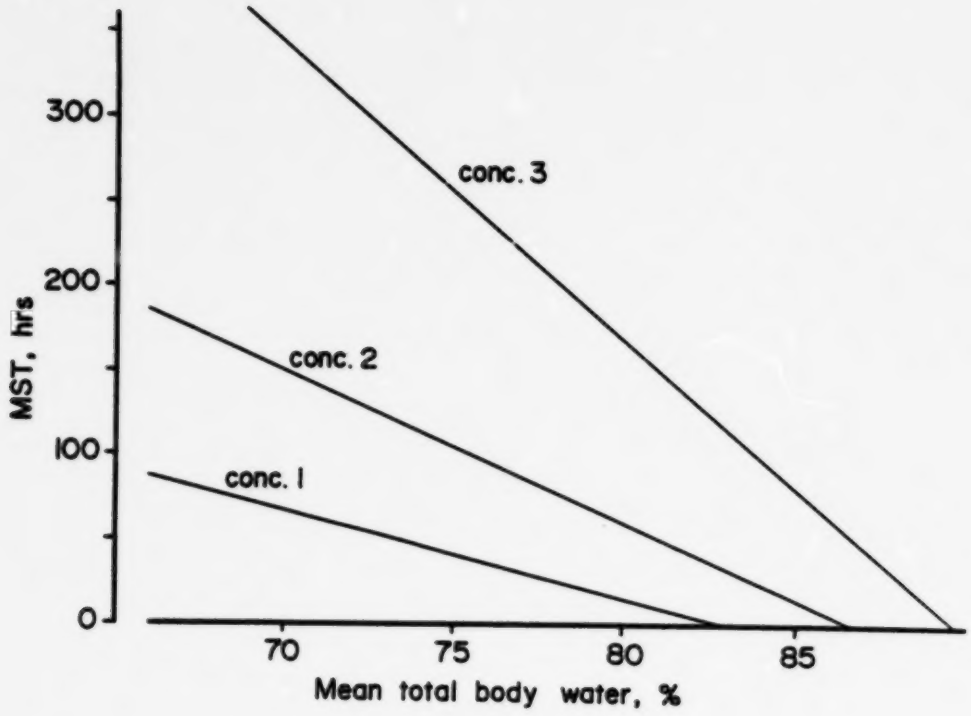

Figure 8. Correlation between Median Survival Time, MST, in 3 concentrations of endrin and mean total body water content for fathead minnows.

Data for linear regression analysis were obtained from group I_1-$VIII_1$ and I_2-$VIII_2$ (Table 10) and group IX-XVI, XV_1 and XVI_1 (Table 11). Endrin concentrations (µg/l) are ranges for corresponding mean values. Correlation coefficient (r), slope (a_1), y-intercept (a_o), standard deviation (s) and number of data points were as follows.

Conc. no.	1	2	3
µg Endrin/l	1.26-1.43	0.63-0.75	0.32-0.39
r	-0.763	-0.769	-0.892
a_1	-5.10	-8.94	-17.38
a_o	424	774	1560
s	20.0	34.5	48.6
n	24	20	16

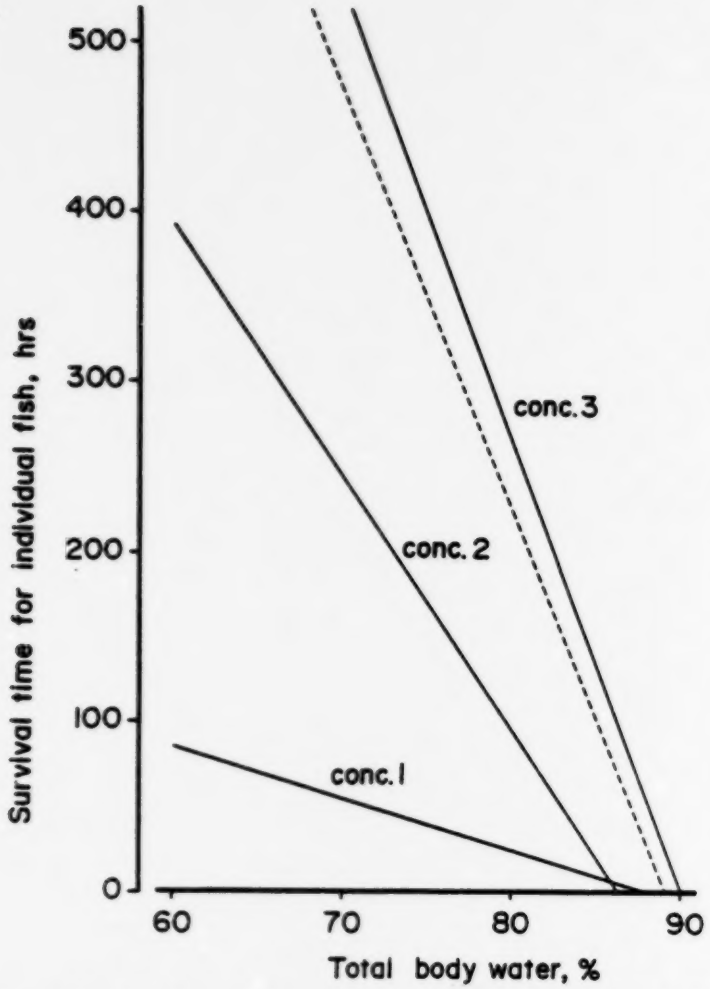

Figure 9. Correlation between survival time in 3 concentrations of endrin and total body water content for individual fish.

Data for linear regression analysis were obtained from group IX-XIII and for conc. 3 also from group XIV-XVI. Endrin concentrations (µg/l) are ranges for corresponding mean values (Table 11). Correlation coefficient (r), slope (a_1), y-intercept (a_o), standard deviation (s) and number of data points were as follows.

Conc. no.	1	2	3	3—
µg Endrin/l	1.37	0.65-0.68	0.35-0.36	0.32-0.37
r	-0.840	-0.758	-0.740	-0.532
a_1	-3.05	-14.86	-26.49	-24.82
a_o	270	1283	2384	2211
s	8.4	42.5	85.9	109.5
n	37	34	34	28
Groups of fish	IX-XIII	IX-XIII	IX-XIII	XIV-XVI

EFFECTS OF ENDRIN ON TOTAL BODY WATER CONTENT

The use of total body water content, when determined post-mortem on intoxicated fishes, in support of the hypothesis that the fat protected the fish from acute intoxication with endrin, assumed that endrin had no or only limited physiological effects on the total body water content. There were, however, some indications in the results presented above were against the validity of such an assumption.

Examining the data in Figure 8 more carefully, it seemed the higher the concentration of endrin was, the lower the mean total body water was. At time zero (i.e., before start of exposure to endrin) the total body water content should be the same independent of toxicant concentration. Total body water content at time zero, estimated from the data in Figure 8, was 83.0, 86.5, and 89.7% at concentrations 1, 2, and 3, respectively. This problem deserves further attention, especially since these data indicated a dose-response relationship.

It was shown in a separate experiment that endrin actually affected the total body water content of fathead minnows. In this experiment the body weights of seven fish were determined before, during and after lethal exposure to two acutely lethal concentrations of endrin (concentration 1 and 2). From the results of this small-scale experiment, presented in Table 13, endrin was shown to affect the body weight of fathead minnows. Variations in body weight during exposure to endrin must be caused mainly by the passage of water between the fish and the environment. Due to the small number of fish that were used and to the fact that only the two highest concentrations were tested, a more thorough interpretation of these results was not possible. However, a dose-dependent effect of endrin on total body water was indicated, in which the lower of the two concentrations (concentration 2) produced a greater increase in body water content than the higher one (concentration 1). Support in favor of this idea was that when the fish were carefully blotted dry after exposure (i.e., according to the procedure which was used for post-mortem determination of body weight in the present study), then the increase in body weight was significantly higher in fish exposed to the lower concentration (concentration 2) compared to the higher one (concentration 1). Further support for the idea that endrin produced a dose-dependent effect on total body water in the fathead minnow is presented in Figures 10, 11 and 12. These data on total body water content also showed that endrin caused a concentration-dependent decrease in total body water content. Thus, in Figure 8 the discrepancy in total body water content at time zero between fish exposed to three different concentrations of endrin, can be explained as a physiological effect of endrin on the osmoregulatory process.

Such an effect of endrin does, however, not rule out the support for the hypothesis of a protective effect of the body lipids on intoxication by endrin. On the contrary the results suggested that in spite of the lowering effect of endrin on total body water a high initial water content, which was indicative of a low lipid content, made the fish more susceptible to endrin.

TABLE 13. EFFECTS OF ENDRIN ON TOTAL BODY WEIGHT

	Period in endrin solution (hrs)							
	0	24		48		48		48
Fish no.	B.W.[a](g)	B.W. (g)	Δ(%)	B.W. (g)	Δ(%)	B.W. (g)	Δ(%)	H_2O (%)
1[b]	3.59	4.21	+17.3	4.13	+15.0	3.89	+8.3	84.3
2	1.87	2.24	+19.8	2.14	+14.4	1.95	+4.3	84.6
3	1.17	1.40	+19.7	1.33	+13.7	1.18	+0.9	83.9
Mean ± S.D.			18.9 ± 1.4		14.4 ± 0.7		4.5 ± 3.7	84.3 ± 0.4
4	3.84	3.60	- 6.3	4.58	+19.8	4.46	+16.1	82.1
5	2.57	2.46	- 4.3	2.95	+14.8	2.92	+13.6	81.2
6	3.67	3.53	- 3.8	4.22	+15.0	4.17	+13.6	81.3
7	1.30	1.32	+ 1.5	1.48	+13.8	1.44	+10.8	81.3
Mean ± S.D.					15.9 ± 2.7		+13.5 ± 2.2	81.5 ±0.4

[a] Body weight measured after blotting the fish dry on paper towels.

[b] Fish No. 1-3 were exposed to 1.7 µg endrin/l and Fish No. 4-7 to 0.9 µg endrin/l.

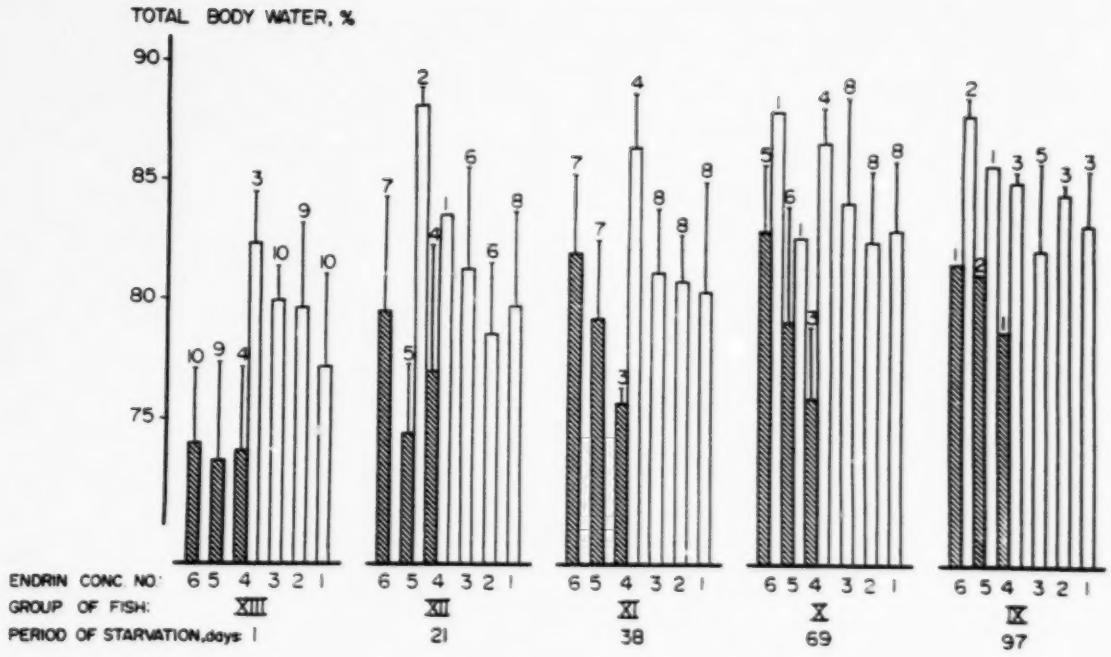

Figure 10. Total body water content of intoxicated and surviving laboratory brood stock fish starved for 1-97 days before exposure to endrin.

Open staples denote intoxicated fish. Shaded staples denote surviving fish after 30 days. Bars denote standard deviation with number of fish (n) above. Average endrin concentrations (µg/l) during exposure were as follows: conc. 1(1.37), 2(0.66), 3(0.35), 4(0.19), 5(0.11), 6(0, control). More detailed information is presented in Table 11.

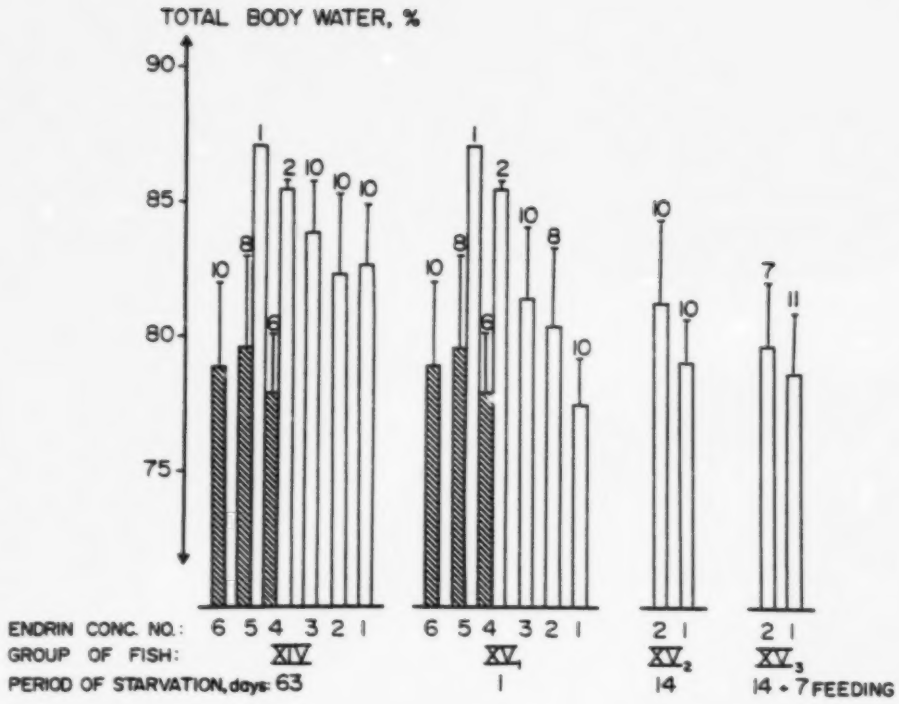
TOTAL BODY WATER, %
90
85
80
75
ENDRIN CONC. NO.: 6 5 4 3 2 1 6 5 4 3 2 1 2 1 2 1
GROUP OF FISH: XIV XV1 XV2 XV3
PERIOD OF STARVATION, days: 63 1 14 14 + 7 FEEDING

Figure 11. Total body water content of intoxicated and surviving pond fish held in the laboratory for 7 months before exposure to endrin. Symbols as in Fig. 10.

Average endrin concentrations for group XIV and XV are presented in Fig. 10. Those for XV_2 and XV_3 are presented in Table 11. Concentration no. 1 was highest concentration and no. 6 was the control.

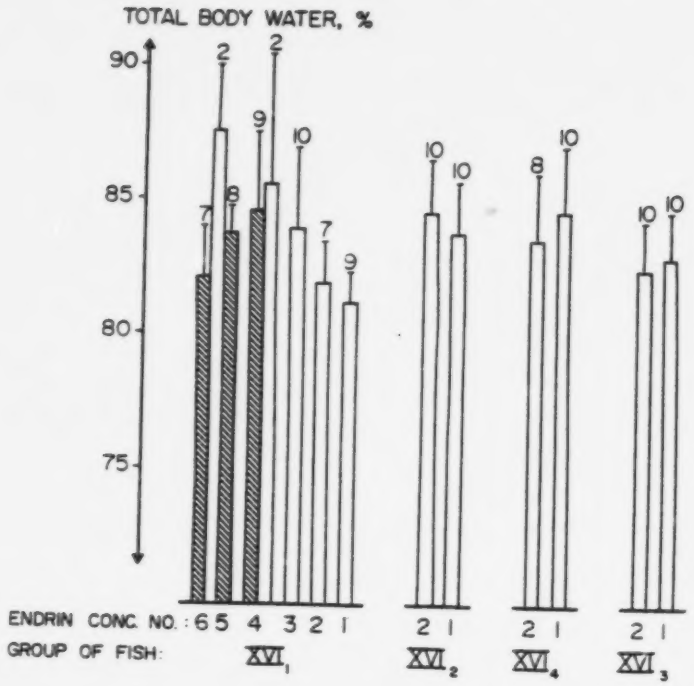

Figure 12. Total body water content of intoxicated and surviving pond fish trapped between 2 and 21 days before exposure to endrin. Symbols as in Fig. 10.

Endrin concentrations (µg/l) are presented in Table 11. Concentration no. 1 was highest concentration and no. 6 the control.

The lowering effect of endrin on total body water in fish has not been reported previously. This effect should be considered during interpretation of experimental data dealing with alterations in blood as well as tissue constitutents (when expressed on a wet weight basis) caused by endrin.

SECTION 7

DISCUSSION

The aim of the present study was to study interactions between nutritional factors and the toxicity of a lipophilic chemical to fish. Endrin was selected as a model toxicant because its toxicity and fate in aquatic organisms were studied quite extensively, providing a great deal of basic knowledge on various toxicological aspects (reviews by Grant, 1976 and by Schimmel et al., 1974).

The results of the present study are discussed from the point of interactions between nutritional factors and the toxicity of endrin. Special attention is focused on the role of lipid metabolism, as I believe that alterations in lipid metabolism and lipid depots are of great importance in the interpretation of results. I have also tried to interpret the ecological implications of the results, and to relate the findings to results from previous studies on interactions between ambient conditions, biological factors, and endrin toxicity to fish.

From the results reviewed in Table G1 (Appendix G) it can be seen that among various ambient conditions, (1) temperature (Katz and Chadwick, 1961; Macek et al. 1969), (2) loading density (Katz and Chadwick, 1961), and (3) the presence of activated charcoal (Brungs et al., 1966) produced significant effects on endrin toxicity to fish. Based on mean values (no confidence limits reported), interactions producing a 2 times or greater variation in acute toxicity (LC50) have also been found for (4) chemical formulation of endrin (Henderson et al. 1959), and for (5) static compared to dynamic test conditions (Lincer et al., 1970.

Among those ambient conditions, which were previously shown to affect endrin toxicity to fish, only those related to renewal of toxicant solution, (2) and (5) above, need to be considered during an interpretation of the results obtained in the present study. Such factors were at least partly responsible for the slightly lower survival times, which were obtained after increasing the flow rate between the first and second series of fathead endrin exposures (Fig. 4 and 5, Table 10). Minor variations in water temperature (Appendix A) and water characteristics (Appendix B) were not considered an important variable in the present study.

Among the ambient conditions studied previously (Table G1, Appendix G), water temperature is probably the one of greatest ecological importance for endrin toxicity to fish. Based on the laboratory toxicity data reported by Katz and Chadwick (1961), a 25-fold increase in acute toxicity (96-hr LC50 for bluegill) was found with an increase in water temperature from 3 to 25°C.

Besides the influence of ambient conditions on endrin toxicity to fish, the influence of certain biological factors have been shown. Among these biological factors, species, population and size as well as developmental stage have been shown to influence endrin toxicity to fish. To examine these results in more detail is beyond the scope of the present report. However, it can not be excluded that lipid content may be an underlying reason for significant variations in endrin toxicity due to species, population, size and developmental stage.

In addition to ambient conditions and biological factors, the period of exposure is certainly of utmost importance for the toxicity of a bioaccumulating chemical like endrin. Reported bioconcentration factors for endrin in fish range between 1,640 (Argyle et al., 1973) and 15,000 (Hermanutz, 1978). Inspite of the high bioconcentration of endrin in fish, the variations between acute toxicity (96-hr LC50) and chronic toxicity (MATC) are surprisingly small, as seen from reported application factor values of 0.46 for fathead minnows (Jarvinen and Tyo, 1978) and 0.30 for flagfish (Hermanutz, 1978).

In the present study three nutritional factors (1) dietary composition, (2) dietary ration level, and (3) starvation, were each shown to influence the acute toxicity of endrin by about 2 times. It is hard to imagine, in a more natural or even in a laboratory situation, how these three nutritional factors could have a simultaneous influence, and thus, produce a combined effect of 8 times (2x2x2) higher susceptibility. A more accurate way to interpret these nutritional interactions is probably to regard them as three separate ways to influence one common factor important for resistance to endrin. This common factor was indicated to be the total body lipid content. Concomitant with a decreased lipid content, there was generally an increased water content in fish (Love, 1970). In the present study an increased total body water content was shown to decrease the resistance to endrin (Fig. 9). This correlation between total body water content was found independent of pre-treatment (Fig. 8).

One ecological implication of the present findings was that laboratory acute toxicity data, achieved in tests on well-fed fish, might underestimate the toxicity of lipophilic chemicals to natural populations of less well-fed fish. The magnitude of this underestimation might be at least 2-fold, when based on 96-hr LC50 values. For a more unbiased and correct interpretation of this finding, parallel toxicity tests with laboratory stock fish and field-collected specimens should be undertaken. Moreover, the influence of temperature must also be considered as temperature interacts strongly with the acute toxicity of lipophilic chemicals like endrin.

Three main factors determine the dose of a lipophilic chemical dissolved in water to fish. These three main factors are (1) concentration, (2) time of exposure, and (3) temperature. The toxic effect of a dose is to a great extent determined by its distribution within the body on an organ as well as a cellular and a subcellular basis. As unionized lipophilic chemicals are transferred over biological membranes by passive processes, their distribution within the body at steady state is determined by the

distribution of the lipids. Considerations of lipid metabolism and lipid distribution then become very important for the understanding of the bioconcentration, biomagnification and effects of lipophilic chemicals in fish as well as in other living organisms.

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APPENDIX A. MEASURED FLOW RATES AND TEMPERATURES DURING TOXICITY TESTS WITH ENDRIN ON FATHEAD MINNOWS

Flow Rates (ml/cycle time of 1 min 48 sec)

Date	Tank no.[a] 1 U	1 L	2 U	2 L	3 U	3 L	4 U	4 L	5 U	5 L	6 U	6 L	Comment
April 9	148	1080[b]	136	980	131	980	126	930	148	1000	108	730	v side[c]
	134	990	134	990	146	1000	132	1030	142	1100	112	830	vv side[c]
May 7	670[b]		850		850		860		910		410		v side
	1060		800		900		860		790		770		vv side
	d										d		
	830												v side
	750												vv side
	d												
May 8	815		800		780		860		870		370		
	940		740		930		840		805		765		

[a] Tank no. 1-6 in decreasing order for toxicant concentrations. Proportional dilution for 1-5 was 1:1. Tank no. 6 was control (no toxicant).

U = upper tanks, L = lower tanks

[b] Flows in lower tanks measured with flow to upper ones shut-off.

[c] V side and vv side relates to duplicate sides

[d] Adjustment in the flow splitting cells performed between measurements. Flow rates in upper tanks, which were drained into the lower ones were increased by means of the flow splitting cells.

APPENDIX A. (Continued -- Water Temperatures (°C)).

Date	Tank no. 1 U	1 L	2 U	2 L	3 U	3 L	4 U	4 L	5 U	5 L	6 U	6 L	Comment
April 23	24.9	26.6	24.8	26.8	24.9	27.0	24.7	27.1	25.2	26.9	23.9	26.8	v side
	24.8	26.7	24.8	26.9	25.1	27.0	24.8	27.1	24.9	27.0	24.0	26.7	vv side
May 1	24.6	26.6	24.7	26.8	24.8	27.1	24.9	27.0	24.9	26.8	24.6	26.5	v side
	24.7	26.6	24.5	26.8	25.1	27.1	24.7	27.1	24.8	27.1	24.4	26.6	vv side
May 4	24.5	26.7	24.5	26.8	24.8	27.0	24.9	27.0	24.8	26.8	24.4	26.5	v side
	24.4	26.6	24.2	26.8	25.0	26.9	24.6	27.0	24.8	27.0	24.4	26.8	vv side
May 7	24.0		24.2		24.4		24.4*		24.4		24.2		
	23.7		24.1		24.6		24.1		24.2		24.0		
May 17	24.7	24.9	25.2	25.2	25.6	25.6	25.5	25.3	25.3	25.1	25.1	25.0	
	24.9	25.1	25.1	24.8	25.4	25.6	25.4	25.3	25.4	25.4	25.0	24.9	

* Temperatures always measured close to the drain. Temperature on incoming water (flow splitting chamber for upper tank divider) was 25.7°C on this occasion compared to 24.4°C for the outlet. This means that the temperature control for the upper tanks in all experiments was approximately within ± 0.7°C with a mean temperature close to 25.1°C. For the lower tanks the increase of flow rate to the upper tanks, which was compensated for by adjusting the temperature control in the headbox (water supply), created a drop in temperature by almost 2°C. Mean and range of temperature for the lower tanks is considered to be 26.0 ± 1°C.

APPENDIX B. ALKALINITY, HARDNESS AND pH OF THE DILUTION WATER

TABLE B1. ALKALINITY, HARDNESS AND PH OF THE WATER SUPPLY, UNTREATED LAKE SUPERIOR WATER

Date	Alkalinity (mg/l as $CaCO_3$)	Hardness (mg/l as $CaCO_3$)	pH	Experiment
January 5	41	45	7.5	Feeding and starvation
January 13	41	45.5	7.3	
January 22	42.5	46.5	7.3	
January 30	42	46	7.3	
February 8	42.5	46.5	7.4	
February 15	42.5	46	7.3	
February 22	42	47	7.3	
March 1	42.5	46.5	7.3	
March 8	44	46.5	7.2	
March 18	43.5	49.5	7.4	
March 23	44	46.5	7.2	
April 1	42.5	45.5	7.3	
April 7	42	44.5	7.3	
April 13	41.5	44	7.2	
April 21	39.5	44.5	7.3	
April 28	40	45.5	7.3	
May 7	40	45.5	7.3	Toxicity test
May 14	41	45	7.4	
May 21	39.5	44	7.5	
June 1	40	43.5	7.5	
June 7	41.5	44	7.6	Termination

TABLE 82. WATER CHARACTERISTICS IN DIFFERENT EXPOSURE CHAMBERS DURING BIOASSAY

Chamber No.	1		2		3		4		5		6	
	v	vv	v	vv	v	vv	v	vv	v	vv	v	vv
Acidity	1.943	1.6665	1.388	1.943	1.665	1.665	2.220	1.388	1.665	1.665	1.943	1.665
Alkalinity, mg/l as $CaCO_3$	42.4	42.4	42.4	42.4	42.4	42.4	42.4	42.4	42.4	42.4	41.4	41.4
Dissolved oxygen, mg/l	8.8	8.4	8.6	8.8	8.4	8.6	8.4	8.4	8.3	8.4	8.4	8.2
Hardness, mg/l as $CaCO_3$	44.5	43.5	43.0	44.0	44.5	44.5	43.0	43.5	43.5	42.5	44.0	44.5
pH	7.73	7.84	7.67	7.30	7.54	7.62	7.73	7.74	7.71	7.70	7.67	7.75

All samples were taken from the middle of upper tanks on May 18, except for dissolved oxygen, which was determined on May 17.

APPENDIX C.

TABLE C1. MEASURED CONCENTRATIONS OF ENDRIN (µg/l) IN THE WATER DURING TOXICITY TESTS WITH FATHEAD MINNOWS

Day No.	Stock solution	Exposure concentration No.				
		1	2	3	4	5
0	213	1.5	.74	.41	.19	.12
1	212	.66	.43	.24	.16	.07
2	212	1.1	.51	.26	.17	.10
3	209	.79	.48	.34	.16	.083
4	206		.68	.36	.19	.11
5						
6						
7	153		.42	.20	.11	.063
8	198					
9						
10						
11	270					
12						
13						
14	202	1.5	.76	.37	.18	.12
15	195	1.3		.33		.092
16	203	1.6	.72	.35	.18	.090
17	195	1.4	.62	.34	.17	.118
18						
19						
20						
21	274	2.1		.44		.131
22						
23	255		.94	.48	.24	.166
24						
25	245	1.7	.90	.43	.23	.128
26						
27						
28						
29	238	1.8	.89	.44	.24	.14

APPENDIX D. FOOD CONSUMPTION AND MORTALITY DURING REARING PERIOD PRIOR TO TOXICITY TESTS

TABLE D1. DAILY FOOD CONSUMPTION, F.C. (g/day/group of fish) AND NUMBER OF DEAD FISH, N.D. FOR SEVEN GROUPS OF FATHEAD MINNOWS FED DIFFERENT DIETS (I-VII). EACH GROUP STARTED WITH 220 1-MONTH-OLD FISH

	Group of fish														
	I		II		III		IV		V		VI		VII		
Day No.	F.C.	N.D.	F.C.	N.D.	F.C.	N.D.	F.C.	N.D.	F.C	N.D.	F.C.	N.D.	F.C.	N.D.	Comments
0	8.1		6.8		7.2		8.4		6.8		7.9		3.6*		Feb. 9, 1979
1	7.2		5.1		4.9		6.8		7.5		6.7		3.6*		
2	5.5		3.8		4.1		5.8		5.1		4.1		3.6*		
3	1.9		1.4		1.0		1.4		1.1		1.4		1.4		Food offered 2 hrs
4	5.9		5.9		5.5		7.1		4.3	1	7.6		3.6	1	
5	4.3		4.8		4.5		7.2		3.3		7.2		48		
6	3.5		5.1		3.6		6.6		2.3		5.3		8.8		
7	5.5		7.0		4.4		7.2		3.8		4.9		8.9		
8	5.4		6.8		6.3		5.6		4.3		4.4		9.6*		
9	4.2		5.3		5.3		5.6		3.9		3.1		4.1		
10															No feeding
11	5.4		3.6		4.0		6.2		4.5		3.5		9.6		

(continued)

TABLE DI. (Continued)

Day No.	Group of fish I F.C.	I N.D.	II F.C.	II N.D.	III F.C.	III N.D.	IV F.C.	IV N.D.	V F.C	V N.D.	VI F.C.	VI N.D.	VII F.C.	VII N.D.	Comments
12	6.4		6.0		6.4		6.3		5.1		5.1		9.4		
13	7.4		6.8		6.0		6.6		5.7		6.5		9.6*		
14	6.7		6.4		6.4		6.3		5.5	1	6.2		9.6*		
15	5.8		6.9		4.8		8.6		4.2		5.4		9.6*		
16	4.4		4.6		3.2		7.1		2.5		3.5		9.5		
17	6.5		6.3		4.6		5.4		4.0	2	3.7		11.9		
18	5.8		7.1		5.7		6.9	1	4.5	1	4.9		12.7		
19	5.2	1	5.9		5.1		6.2		3.3		5.8		14.2		
20	5.3		5.6		3.7		5.8		3.8		5.3		14.2		
21	6.0	1	7.0		6.9		7.6		4.3		5.7		14.4*		
22	6.0		7.9		7.8		7.4		5.3		5.3		14.4*		
23	3.8		6.3		6.4		5.4		3.9		4.3		13.0		
24	6.6		7.1		6.2	1	7.3		3.9		4.2		14.2		
25	6.5		8.6		7.2		7.6		5.5		7.9		13.3		

(continued)

TABLE D1. (Continued)

Day No.	Group of fish I F.C.	I N.D.	II F.C.	II N.D.	III F.C.	III N.D.	IV F.C.	IV N.D.	V F.C	V N.D.	VI F.C.	VI N.D.	VII F.C.	VII N.D.	Comments
26	3.0	2	6.7	1	6.0		6.8		4.7	1	4.2		12.8		
27	6.6	3	7.1	2	5.3	1	6.7	2	3.7	1	3.8		13.1		
28	6.8	2	7.6		6.3		6.8		5.1	1	6.1		9.6		
29	5.4		7.0		7.1		5.7		5.6		5.7		9.6		
30	4.1		5.1		6.0		6.0		2.7		3.5		8.9		
31	6.6		8.0	3	7.6	1	6.5	1	4.1	3	5.2		9.4		
32	8.0	4	8.6	2	7.4		8.4		5.5	3	5.8		9.6		
33	7.2	1	8.4		6.4	1	8.1		5.4		9.4		14.2		
34	7.3	2	9.1		7.5		7.9	1	4.9	1	7.7		9.6		
35	5.7	1	6.7		6.0	2	7.3		4.4		8.1		9.5		
36	6.8		6.1		6.3		9.0		3.8		4.0		9.6		
37	5.7		7.3	2	6.1	1	5.2	4	4.8		5.7	1	9.5		
38	8.3	1	8.8	1	6.6	1	6.9	3	3.9	2	5.9	2	14.0		
39	8.2	1	9.0	2	7.3	1	7.8	1	6.0	1	7.6	1	13.7		

(continued)

TABLE D1. (Continued)

Day No.	Group of fish I F.C.	I N.D.	II F.C.	II N.D.	III F.C.	III N.D.	IV F.C.	IV N.D.	V F.C	V N.D.	VI F.C.	VI N.D.	VII F.C.	VII N.D.	Comments
40	8.2	1	8.5	1	7.6		7.9		5.7		7.9		13.6		
41	8.0	2	8.5		6.0	2	7.7		4.3	2	6.3	1	13.1		
42	7.9	1	6.1		7.5	3	6.4	1	3.9		6.0		12.7		
43	7.2		6.9	1	5.2		5.3		2.5	1	4.8	1	12.4		
44	7.2		8.5		7.4		7.4		5.6		6.8		12.5		
45	8.1		8.8	1	7.5		8.7	1	3.7		5.7		13.3		
46	8.1		8.6	1	7.6	1	8.3		4.7	1	7.0		13.1		
47	6.8		8.1		6.8	1	8.1	1	6.2	3	7.2		12.3		
48	6.8		7.0	1	5.2	4	8.0		3.3		6.0	2	11.6		
49	7.2		7.1	1	7.1	1	8.2		5.6	1	7.8		11.2		
50	7.3		7.3		7.1	1	7.6		5.2	2	7.5		8.3		
51	7.1		7.0	1	7.2	1	7.8	1	4.9		7.4	1	9.7		
52	8.0	1	8.0	1	7.2	3	8.1	1	4.6	1	7.3	1	14.2*		
53	8.1	1	8.6		6.3		8.5		5.5	1	7.6	1	12.0*		

(continued)

TABLE D1. (Continued)

Day No.	Group of fish I F.C.	I N.D.	II F.C.	II N.D.	III F.C.	III N.D.	IV F.C.	IV N.D.	V F.C	V N.D.	VI F.C.	VI N.D.	VII F.C.	VII N.D.	Comments
54	7.8	1	8.2		6.6		8.1	1	4.3		7.7	1	8.1		
55	6.9	1	7.9		6.8		8.1	1	3.8		7.3	2	8.0		
56	7.1		8.1		5.7		7.5		3.7		6.7		8.7		
57	8.1		6.6		6.5		8.4		3.9	1	7.3		9.5		
58	6.7	2	7.7	1	6.2		8.4	1	4.4		6.9		9.5		
59	7.5		8.2	1	7.5	2	8.6		5.9		8.3	2	10.4		
60	7.7		8.5		7.8		8.5		6.4		8.1		8.9		
61	6.0		6.4	1	6.6		6.5	1	4.4	2	6.5		4.8		
62	8.1		7.1	2	4.9	1	7.5	2	5.1		7.0	1	9.4		
63	7.7	2	8.3	1	6.6	1	8.0	1	5.0	3	8.0		8.5		
64	7.5		8.2		8.3	1	9.0		4.5		7.3	2	9.5		
65	8.1		7.0		4.8		8.9		4.8	1	7.5		10.8		
66	8.3		7.2	2	7.3	1	9.2		5.6	1	9.3	3	8.4		
67	7.5		7.6	1	6.5		8.7	1	5.2		8.8		8.3		
68	6.8	1	7.9	2	8.5	1	7.6		5.4	2	8.7	1	8.2		

(continued)

TABLE DI. (Continued)

Day No.	Group of fish I F.C.	I N.D.	II F.C.	II N.D.	III F.C.	III N.D.	IV F.C.	IV N.D.	V F.C	V N.D.	VI F.C.	VI N.D.	VII F.C.	VII N.D.	Comments
69	7.9		8.2		7.6		8.4		5.0		8.6	2	8.6		
70	8.6	1	7.2		7.3	1	8.2	1	5.4	2	8.2	1	8.9		
71	8.2		7.5	1	6.3	1	7.6		6.9		8.1	1	9.3	1	
72						1		1		1		2			No food off.
73						1		1							No food off.
74	7.3	4	9.0	4	9.0		9.2	1	8.2		7.9		9.3		
75	8.7	2	8.3		7.5		8.8	1	6.5		7.5		9.1		
76	6.7	1	7.5	1	7.2		8.7	1	6.8	1	7.8		8.5		
77	7.2		5.7		6.8	2	8.2	1	6.3		7.9	1	7.0		
78	8.0	1	6.0	2	7.6	3	6.9		7.2		8.2	1	8.9		
79				2								1			No food off.
80	4.8	1	4.5	3	4.5		6.8	3	2.8		5.9		7.3		
81	5.6	4	7.6	5	5.4	1	5.0		3.5	2	7.4	5	4.2		
82	6.7		6.3		5.2	1	4.8		3.4	1	6.5		4.2		

(continued)

TABLE D1. (Continued)

Day No.	Group of fish I F.C.	I N.D.	II F.C.	II N.D.	III F.C.	III N.D.	IV F.C.	IV N.D.	V F.C	V N.D.	VI F.C.	VI N.D.	VII F.C.	VII N.D.	Comments
83	4.7	1	6.7	1	6.4		5.0		3.1		6.3		4.8*		
84	4.5	1	7.0	1	5.8	1	4.4		3.7		6.2	2	6.6		
85		1								2		1			No food off.
86	5.7		8.1		8.4		7.7		6.1	1	7.5	1	5.7		
87	4.2	3	5.5		6.3	1	4.9		3.4		6.8	1	4.7		
88	5.9		6.6	2	7.2	2	5.6		4.3		7.6		7.2		
89	5.4		6.9	2	6.8	3	4.2	1	4.5	1	7.0	1	4.8		
90	5.5		4.8		6.0		3.6		3.1		3.9		4.7		
91	4.6		5.2	1	5.7	2	4.5	1	3.8	1	6.8	1	4.7		
92	5.2		6.4	1	5.4	2	4.7	1	4.2		6.2		8.6		
93				2		3		1							No food off.
94	1.9		5.4		5.0	2	4.1	4	2.6	1	6.3		4.7		
95	1.1		2.8		2.5	1	2.3		1.7		2.0		8.5		

(continued)

TABLE DI. (Continued)

Day No.	Group of fish I F.C.	I N.D.	II F.C.	II N.D.	III F.C.	III N.D.	IV F.C.	IV N.D.	V F.C	V N.D.	VI F.C.	VI N.D.	VII F.C.	VII N.D.	Comments
96	1.1		3.1	1	2.5		3.0		2.2	2	3.0		7.9		
97	1.0		2.7		2.6	1	2.6	1	1.8		2.4		4.0		
98	1.3		2.9	1	3.1	2	2.8	1	1.9		2.7		4.4		
99	1.7		3.8		3.3		2.2		2.3		3.1		3.7		
100	1.5		3.3		3.3		2.8	2	2.8		3.7		4.0		
	+		+		+		+		+		+		+		
1-100	52		62		64		48		54		45		2		

* All food consumed

APPENDIX E

BODY WEIGHT, WATER CONTENT AND SURVIVAL TIME FOR FATHEAD MINNOWS SUBJECTED TO STARVATION PRIOR TO TOXICITY TESTS WITH ENDRIN

TABLE E1. INITIAL BODY WEIGHT (g) OF FATHEAD MINNOWS SUBJECTED TO STARVATION*

Group No.	IX	X	XI	XII	XIV
Mean	4.00	4.75	2.56	2.32	2.37
S.D.	1.80	1.97	0.93	0.98	1.07
No. of Fish (N)	50	50	50	50	60
Range	0.70-7.10	1.80-11.90	1.43-6.60	1.12-5.76	1.07-4.68

*Initial body weights were not determined for groups XIII, XV and XVI, which were subjected to toxicity tests within 24-48 hours of starvation and acclimation.

TABLE E2. BODY WEIGHT (g) AND BODY WATER CONTENT (%) OF FATHEAD MINNOWS KILLED BY STARVATION PRIOR TO TOXICITY TESTS

Group No.	IX	X	XI
Body Weight			
Mean	2.01	1.86	2.40
S.D.	0.63	0.39	0.57
Range	0.55-3.71	1.37-2.18	1.99-2.80
Water Content			
Mean	86.8	86.0	83.7
S.D.	2.2	1.7	1.7
Range	81.8-90.0	84.4-88.4	82.5-84.9
No. of Fish (n)	24	4	2

TABLE E3. BODY WEIGHT (BW), BODY WATER CONTENT (H_2O) AND SURVIVAL TIME OF FATHEAD MINNOWS EXPOSED TO 5 CONCENTRATIONS OF ENDRIN AND CONTROLS

Group IX - Fish starved for 95 days prior to exposure

	Concentration No.											
	1		2		3		4		5		6	
Survival time (hrs)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)
6.6	3.37	84.0										
14.7	4.24	82.1	2.22	85.6	3.10	85.6						
	4.69	86.6	2.01	85.1								
			0.64	85.9								
22.6					1.95	86.2						
40.7							2.47	85.8				
64.3							2.71	85.6				
96.1					1.57	85.4			0.45	86.7	1.97	89.3
128.5											1.88	88.3
152.7							3.79	86.5				
191.6					1.64	79.9						
419.1					1.21	78.5						
>696							0.59	79.7	2.57	82.1	1.55	82.6
									1.06	82.1		

Continued

TABLE E3. (Continued)

Group X - Fish starved for 59 days prior to exposure

Survival time (hrs)	Concentration No. 1 B.W. (g)	1 H_2O (%)	2 B.W. (g)	2 H_2O (%)	3 B.W. (g)	3 H_2O (%)	4 B.W. (g)	4 H_2O (%)	5 B.W. (g)	5 H_2O (%)	6 B.W. (g)	6 H_2O (%)
1.5			2.14	85.0								
6.5	3.93	86.3										
	4.93	85.4										
	3.53	85.6										
14.8	3.44	81.7	2.12	85.8	2.12	88.7	3.58	86.6				
	3.30	84.5			2.76	87.0						
22.7	3.08	80.5	4.17	86.1	5.55	87.2						
	2.09	81.3										
30.7	3.01	78.1					3.02	88.7				
40.2							3.82	85.6				
51.9			4.38	81.5								
72.3			6.04	82.1								
84.2			2.29	80.3	4.28	86.4						
			3.00	81.3	5.42	85.1						
128.3			3.45	77.4								
251.2					7.34	83.4						
319									4.99	82.6		
343.8					3.11	78.8						
419.3					1.76	76.3	2.34	85.5				
443.3											2.39	87.9
>696							2.76	72.5	2.56	78.9	1.98	82.8
							1.62	77.8	2.28	70.6	2.31	86.6
							1.78	77.5	2.27	84.1	2.75	84.7
									1.89	79.9	1.78	79.8
									1.87	77.5	2.34	80.8
									1.39	83.5		

Continued

TABLE E3. (Continued)

Group XI - Fish starved for 38 days prior to exposure

Survival time (hrs)	Concentration No. 1 B.W. (g)	1 H_2O (%)	2 B.W. (g)	2 H_2O (%)	3 B.W. (g)	3 H_2O (%)	4 B.W. (g)	4 H_2O (%)	5 B.W. (g)	5 H_2O (%)	6 B.W. (g)	6 H_2O (%)
6.5	2.77	87.0										
15	2.06	82.0										
	1.48	83.1										
	1.92	84.9										
22.8	1.66	78.3	1.50	84.7								
30.3	2.81	76.2	1.97	81.2								
	1.32	77.6										
39.6					1.69	86.4						
47.2			1.52	90.3								
			1.33	83.5								
64	1.62	73.3										
72			1.73	78.6								
			1.88	80.3								
			1.69	77.5								
107			1.68	78.6								
127.8			1.38	78.3	1.43	83.9						
215.3					0.96	83.3						
251							1.24	89.5				
287					1.42	81.0						
					1.90	80.5						
					1.27	78.0						
					2.52	80.6						
343							1.18	85.6				
488							1.14	84.2				
516					1.85	77.3						
>696							1.60	75.0	2.88	84.7	1.02	81.4
							1.35	75.6	1.67	77.8	1.33	85.6
							1.02	76.5	1.88	78.2	1.36	81.6
									2.47	74.9	1.18	75.4
									1.41	82.3	0.95	83.2
									1.93	79.3	1.15	81.7
									1.33	77.4	1.30	84.6

Continued

TABLE E3. (Continued)

Group XII - Fish starved for 21 days prior to exposure

Survival time (hrs)	Concentration No. 1 B.W. (g)	1 H_2O (%)	2 B.W. (g)	2 H_2O (%)	3 B.W. (g)	3 H_2O (%)	4 B.W. (g)	4 H_2O (%)	5 B.W. (g)	5 H_2O (%)	6 B.W. (g)	6 H_2O (%)
6.5	3.05	85.6										
15	1.10	81.8										
	3.38	84.6										
22.7	1.97	74.6										
	2.34	75.6										
30.4	1.84	78.8										
	1.84	78.8										
39.2			5.31	82.1								
54.8	2.61	78.2										
83.5			2.53	81.8								
			2.36	78.4								
110							1.75	80.0				
179			1.72	77.9	0.81	87.7						
202.8			1.26	77.0								
215.3					1.54	77.9						
237.5			1.94	74.2								
250					2.47	85.4						
					2.57	79.0						
275					2.46	80.5						
					2.16	77.3						
551									2.21	88.7		
									1.38	87.7		
566							1.65	83.6				
>696							1.92	74.0	1.53	79.1	2.35	85.5
							1.09	84.4	1.34	73.9	1.22	78.7
							1.40	77.1	1.91	71.2	1.92	75.5
							1.20	72.5	1.23	73.2	0.80	76.3
									1.22	74.6	1.19	73.9
											1.07	85.0
											1.08	82.4

Continued

TABLE E3. (Continued)

Group XIII - Fish starved for 24 hrs prior to exposure

Survival time (hrs)	Concentration No. 1 B.W. (g)	1 H_2O (%)	2 B.W. (g)	2 H_2O (%)	3 B.W. (g)	3 H_2O (%)	4 B.W. (g)	4 H_2O (%)	5 B.W. (g)	5 H_2O (%)	6 B.W. (g)	6 H_2O (%)
6.5	4.76	84.7										
15			4.92	85.8								
22.6	3.42	77.8										
29.6	3.06	75.8	2.98	82.9								
	1.53	81.7										
	1.78	76.4										
34.9	1.84	76.1										
39.5	1.36	77.9	1.70	79.4								
	1.84	76.6										
46.8							4.40	84.8				
54.6	2.42	74.8	1.37	80.3								
63.4	2.75	70.2										
83.7					2.00	81.5						
95.3			1.78	73.6								
135			2.95	81.7								
			2.72	77.6								
179					5.25	79.6						
					1.17	76.9						
204			1.69	77.5								
237			1.33	78.2								
250					1.28	80.5						
					1.57	80.3						
					2.04	80.9						
275					3.17	78.9						
					1.92	78.6						
382							2.27	80.6				
465							4.39	80.9				
493					1.09	80.7	2.03	89.7				
>696							2.42	70.2	0.92	71.7	1.29	77.5
							1.69	72.8	1.46	72.6	1.66	69.9
							1.49	73.2	4.99	78.2	1.14	71.1
							1.72	78.5	1.98	72.7	1.59	73.6
									0.89	71.9	1.49	72.5
									1.93	75.1	0.87	80.5
									0.97	79.4	1.72	73.3
									1.35	65.2	2.16	72.7
									1.27	72.4	1.26	73.0
											1.24	75.8

Continued

TABLE E3. (Continued)

Group XIV - Pond-fish starved for 53 days prior to exposure

	Concentration No.											
	1		2		3		4		5		6	
Survival time (hrs)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)
6.4	2.63	86.8										
	2.69	84.8										
15	2.06	81.6	0.48	85.4								
	2.34	81.6	1.17	85.5								
	1.45	82.8										
	2.57	82.5										
	3.49	85.1										
	1.62	80.2										
22.6			1.31	84.0	0.97	85.6						
30.1	1.18	79.7	1.73	80.3	2.92	83.2						
			1.13	83.2								
39.3			1.39	84.9								
			1.01	84.2								
47			1.31	78.6								
54.2	1.15	81.7			2.79	83.5						
71.7			1.87	79.7								
			1.47	77.6								
95.3							1.20	88.3				
132					1.93	82.4						
					1.21	83.5						
					1.04	86.5						
155					3.02	86.1						
					0.94	84.0						
179					1.18	83.9						
295							3.35	85.1				
384									0.48	87.5		
472					1.25	80.0						
534									1.63	83.4		
									1.40	83.6		
>696							0.50	82.0	0.74	82.4	0.60	85.0
							1.00	85.0	1.50	80.7	1.41	85.8
							0.99	84.8	0.93	83.9	0.95	84.2
									1.78	82.6	2.36	86.4
									1.11	81.1	0.98	82.7
									2.56	80.1	2.72	80.5
											1.11	79.3

Continued

TABLE E3. (Continued)

Group XV_1 - Pond-fish starved for 24 hrs prior to exposure

	Concentration No.											
	1		2		3		4		5		6	
Survival time (hrs)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)	B.W. (g)	H_2O (%)
4.8	4.10	79.8										
	2.78	78.1										
15.3	3.78	77.0										
	2.47	77.7										
	2.88	79.2										
	2.45	73.5										
	3.88	77.8										
	1.92	77.1										
	1.81	77.9										
	0.99	76.8										
61			5.00	81.4								
			1.69	83.4								
			2.89	79.9								
			1.30	84.6								
72			1.64	76.2								
85			5.81	81.2								
99			1.52	77.0								
			3.36	79.5								
180					2.87	82.9						
191					5.05	81.6						
229					2.68	78.5			2.49	87.1		
240					1.17	83.8						
273					2.20	77.7						
421					1.42	79.6						
445							2.30	85.7				
							1.90	85.3				
527					2.37	78.9						
>696							2.05	76.6	1.58	79.1	2.23	73.1
							4.35	79.8	1.02	78.4	4.62	82.0
							1.01	79.2	2.49	84.7	1.77	77.4
							1.16	79.3	1.26	75.4	2.51	81.7
							1.89	74.1	1.01	77.2	2.07	76.3
							2.47	78.5	1.38	79.0	1.88	78.2
									1.80	79.4	0.80	83.8
									1.20	77.5	1.79	78.2
									1.90	85.8	1.07	80.4
											1.94	77.8

Continued

TABLE E3. (Continued)

Group XVI_1 - Pond-fish trapped 2 days prior to exposure

Survival time (hrs)	Concentration No. 1 B.W. (g)	1 H_2O (%)	2 B.W. (g)	2 H_2O (%)	3 B.W. (g)	3 H_2O (%)	4 B.W. (g)	4 H_2O (%)	5 B.W. (g)	5 H_2O (%)	6 B.W. (g)	6 H_2O (%)
5.5	1.78	80.9	2.39	82.4	1.70	81.8						
	2.89	83.7										
	0.90	81.1										
11.7	1.36	80.1										
	1.00	82.0										
16.4	2.90	81.0	3.11	83.0								
	1.97	80.7	1.47	81.6								
	1.26	81.0	1.55	83.9								
	1.10	80.0	1.87	81.8								
23.1			4.02	81.6								
39			2.92	79.1	1.20	81.7						
					1.32	84.1						
60					1.38	87.0						
					1.57	82.8						
					1.45	81.4						
72					2.09	80.4						
108					1.91	84.3						
					1.51	90.1						
155					1.76	85.8						
228							0.90	82.2				
373											0.56	89.3
501							1.00	89.0			1.55	85.8
>696							0.60	86.7	0.90	83.3	0.67	85.1
							1.03	89.3	0.74	83.5	1.02	83.1
							1.05	84.8	0.59	83.1	0.62	80.6
							0.93	78.5	1.51	82.1	0.67	83.6
							0.59	83.1	0.55	85.5	1.45	80.7
							0.63	84.1	1.06	84.0	0.69	81.2
							0.54	85.2	1.87	34.0	0.60	80.0
							0.43	86.0	2.32	84.1		
							1.33	83.5				

Continued

TABLE E3. (Continued)

Group XV_2 - Group XV_1 starved 14 days prior to exposure

Survival time (hrs)	Concentration No. 1 B.W. (g)	Concentration No. 1 H_2O (%)	Concentration No. 2 B.W. (g)	Concentration No. 2 H_2O (%)
18.5	1.66	81.3	1.36	86.8
	1.25	81.6		
	1.02	78.4		
24.2	1.50	78.7		
27.0	1.93	77.7		
	2.16	79.2		
	1.28	78.1		
30.7	1.62	80.2		
	2.02	78.7		
48.2	1.31	76.3	2.85	82.1
			3.08	81.2
			3.58	86.0
			1.45	79.3
49.0			2.02	79.2
65.5			3.33	80.2
81			1.84	79.3
98			2.16	76.4

Group XV_3 - Group XV_2 after 7 days feeding prior to exposure

Survival time (hrs)	Concentration No. 1 B.W. (g)	Concentration No. 1 H_2O (%)	Concentration No. 2 B.W. (g)	Concentration No. 2 H_2O (%)
13.4	1.66	77.7		
	1.91	78.0		
	4.37	84.2		
	3.11	77.2		
23.0	2.93	79.5		
25.5	1.81	75.7		
29.8	3.66	78.2		
	3.81	78.2		
	2.03	79.8		
40.3			3.36	78.3
48.6	1.88	77.1	4.74	81.9
	2.20	78.6	1.99	78.4
			3.61	83.1
			2.99	77.3
61.3			2.64	77.3
81.7			2.01	81.1

Continued

TABLE E3. (Continued)

Group XVI_2 - Group XVI_1 starved 14 days prior to exposure

Survival time (hrs)	Concentration No. 1 B.W. (g)	Concentration No. 1 H_2O (%)	Concentration No. 2 B.W. (g)	Concentration No. 2 H_2O (%)
9.8	1.46	84.9	0.79	86.1
	1.78	84.3	1.29	84.5
	1.19	86.6		
	1.34	83.6		
	1.96	82.7		
	1.23	82.9		
	1.16	85.3		
	0.92	84.8		
13.5	2.50	82.8		
17.8			1.71	81.9
			1.15	86.1
			0.96	84.4
			1.28	84.4
			0.71	83.1
23.5			1.74	86.2
27			0.93	87.1
41.2			2.30	81.3

Group XVI_3 - Group XVI starved 21 days prior to exposure

Survival time (hrs)	Concentration No. 1 B.W. (g)	Concentration No. 1 H_2O (%)	Concentration No. 2 B.W. (g)	Concentration No. 2 H_2O (%)
12.5	1.40	88.6	2.96	81.8
	3.35	83.6	3.94	82.7
	3.65	79.7	2.80	86.1
	2.31	83.1	1.67	85.6
	3.08	86.0	1.96	82.7
	2.78	84.2	1.94	87.1
	1.70	85.3		
	2.38	85.7		
	1.75	83.4		
	1.10	86.4		
22			2.63	80.2
29			3.53	81.9

Continued

TABLE E3. (Continued)

Group XVI_4 - Group XVI_1 fed for 7 days prior to exposure

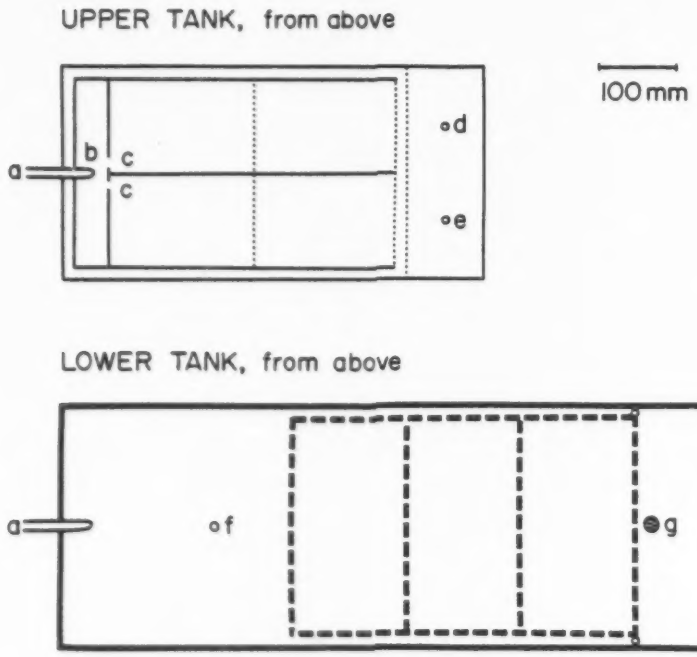
Survival time (hrs)	Concentration No. 1 B.W. (g)	Concentration No. 1 H_2O (%)	Concentration No. 2 B.W. (g)	Concentration No. 2 H_2O (%)
12.8	3.38	82.0		
	2.96	83.1		
	3.21	84.1		
	1.61	84.5		
	1.59	81.1		
23.9	2.34	80.3		
	1.84	83.2		
30	1.76	84.7	2.54	83.5
	3.59	80.8	1.82	85.8
			2.16	84.3
			2.51	80.5
			1.11	82.0
38.8			2.13	82.6
			2.42	80.2
48.5			2.79	81.0
			3.16	82.9
59			3.87	81.4

APPENDIX F.

POSITION OF EXPERIMENTAL GROUPS OF FISH IN SCREENED COMPARTMENTS DURING TOXICITY TESTS.

A system for toxicant exposure previously used for chronic life-cycle tests (Holcombe et al. 1976) was modified slightly to fit the experiments. Each tank was divided by screens according to Fig. F-1. Exposure chambers for experimental groups of fish (I-XVI) were randomized. The positions for various groups in experiment 1 and 2 are presented in Figure F-2.

Figure F-1. Arrangement of testing tanks used for toxicity tests with 16 pre-treated groups of fish.

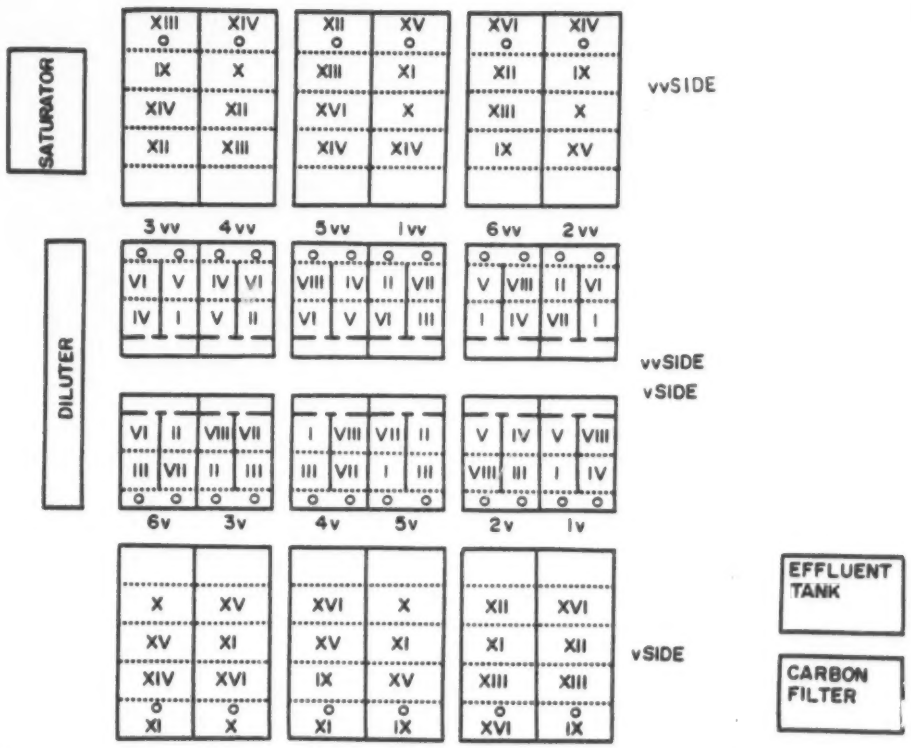


a. Water fed from flow splitting chamber of the diluter.
b. Flow-splitting chamber.
c. Notches for flow-splitting.
d. Drain to lower tank. Height of standpipe 130 mm.
e. Safety drain. Height of standpipe 140 mm.
f. Water fed from upper tank drain (d).
g. Drain to holding tank and carbon filter. Height of standpipe 300 mm.

Material: (———) glass
(▬▬▬) stainless steel
(———) screen, stainless steel, mesh 40, wire 0.40 mm
(▬ ▬ ▬ ▬) screen, stainless steel, mesh 4, wire 0.88 mm.

Volumes: Total upper tank: 20 l Total lower tank: 81 l
Exposure chamber: 3 l Exposure chamber: 14 l.

Figure F-2. Positions of experimental groups of fish during exposure to endrin.



1v to 6v and 1vv to 6vv refer to toxicant concentrations from the diluter. 1 was the highest concentration and concentrations 2 to 5 were achieved by progressive 1:1 dilution in the diluter. 6 was the control. Roman numerals I-VIII indicate the diet-treated groups of fish which were exposed to the upper tanks, and IX-XVI indicate the groups of adult starved fish exposed in the lower tanks.

APPENDIX G.

SUMMARY ON PREVIOUS FINDINGS ON ENDRIN TOXICITY TO FISH

In the late 1950's the extreme toxicity of endrin to fish was reported by Iyatomi et al. (1958) and Henderson et al. (1959). During the 1960's the implications of this extreme toxicity to fish was realized in studies related to the Mississippi River fish kills (Mount and Putnicki, 1966).

Lethal blood concentrations of endrin in two species of fish were established (Mount et al., 1966; Brungs and Mount, 1967). Studies were undertaken on bioconcentration, distribution and/or depuration (Mount, 1962; Bennett and Day, 1970; Argyle et al., 1973; Jackson, 1976) and more recently on biomagnification (Jarvinen and Tyo, 1978). In addition the physiological mechanism of toxic action was studied (Mount, 1962; Colvin and Phillips, 1968; Cutcomp et al., 1971; Eller, 1971; Davis et al., 1972; Grant and Mehrle, 1970, 1973; Cutcomp, 1974).

The toxicity of endrin to fish became extraordinarily complicated, when fish populations in heavily sprayed areas were found to be much more resistant to endrin and other pesticides than previously unexposed fish (Boyd and Ferguson, 1964). Since then various aspects of this resistance phenomenon to endrin in fish has been studied quite extensively (Ferguson and Bingham, 1966; Ferguson et al., 1966; Ludtke et al., 1968; Kynard, 1974; Fabacher and Chambers, 1971, 1976; Wells and Yarbrough, 1972; Yarbrough and Coons, 1974; Yarbrough and Wells, 1971.

Interactions between endrin toxicity to fish and ambient conditions like temperature, hardness, salinity and suspended solids, as well as with biological factors like species, size and developmental stage were also the subject of several studies. The results of these studies are summarized in Table G1.

TABLE G1. SUMMARY OF REPORTED INTERACTIONS[a] BETWEEN AMBIENT FACTORS AND ENDRIN TOXICITY TO FISH

Ambient factor	Range	Species	Response variable	Maximum mean interaction[b]	Reference
Hardness,	400 and 20 mg/l as $CaCO_3$				
Alkalinity, and	360 and 18 mg/l as $CaCO_3$	Fathead minnow	96-hr LC50	1.3/1.0 = 1.3	Henderson et al., 1959
pH (simultaneously)	8.2 and 7.4				
Hardness,	149 and 300 mg/l as $CaCO_3$				
Alkalinity, and	79 and 251 mg/l as $CaCO_3$	Fathead minnow	96-hr LC50	0.47/0.45 = 1.0	Brungs et al., 1966
DO (simultaneously)	5.9 and 7.6				
Salinity	5-27 °/oo	Marine threespine stickleback	48-hr LC50	2.55/1.74 = 1.5	Katz and Chadwick, 1961
	5-27 °/oo	Marine threespine stickleback	96-hr LC50	1.20/1.65 = 0.7	Katz and Chadwick, 1961
Suspended solids	0-50.4 mg/l	Fathead minnow	96-hr LC50	0.52/0.37 = 1.4	Brungs et al., 1966
Activated carbon	0 and 11.7 mg/l	Fathead minnow	96-hr LC50	18.2/0.45 = 40.4 (total endrin)	Brungs et al., 1966
	0 and 11.7 mg/l	Fathead minnow	96-hr LC50	0.66/0.45 = 1.5 (dissolved endrin)	Brungs et al., 1966
Temperature	3-25°C	Bluegill	96-hr LC50	8.25/0.33 = 25.0	Katz and Chadwick, 1961
	12.7-23.8°C	Bluegill	24-hr LC50	2.8/0.8 = 3.50	Macek et al., 1969
	12.7-23.8°C	Bluegill	96-hr LC50	0.61/0.37 = 1.64	Macek et al., 1969
	1.6-12.7°C	Rainbow trout	24-hr LC50	15/2.8 = 5.35	Macek et al., 1969
	1.6-12.7°C	Rainbow trout	96-hr LC50	2.5/1.1 = 2.27	Macek et al., 1969
Formulation	Wettable powder, technical in acetone, or emulsible concentration	Fathead minnow	96-hr LC50	1.9/0.56 = 3.4	Henderson et al., 1959
	with (1:1) or without methyl parathion	Largemouth bass	24-hr LC50	0.48/0.49 = 1.0	Febacher, 1976
	with (1:1) or without methyl parathion	Largemouth bass	96-hr LC50	0.37/0.27 = 1.4	Febacher, 1976

TABLE G1. (Continued)

Ambient factor	Range	Species	Response variable	Maximum mean interaction[a]	Reference
Detergent (LAS)	0 and 1.0 mg/l	Fathead minnow	48-hr LC50	0.37/0.25 = 1.5	Solon et al., 1967
	0 and 1.0 mg/l	Fathead minnow	96-hr LC50	0.25/0.24 = 1.0	Solon et al., 1967
Loading density of fish in static tests	0.6-0.15 g/l	Bluegill	72-hr LC50	2.70/0.95 = 2.8	Katz and Chadwick, 1961
Loading density of fish in static tests	0.6-0.15 g/l	Bluegill	96-hr LC50	1.10/0.60 = 1.8	Katz and Chadwick, 1961
Static vs. dynamic test	10 l (static) or 12.5 ml/min (dynamic)	Fathead minnow	48-hr LC50	0.77/0.57 = 1.4	Lincer et al., 1970
Static vs. dynamic test	10 l (static) or 12.5 ml/min (dynamic)	Fathead minnow	96-hr LC50	0.77/0.39 = 2.0	Lincer et al., 1970

[a] Most of these interactions between endrin toxicity and ambient factors were considered as insignificant by the investigators. Significant interactions were only evident for temperature (Katz and Chadwick, 1961 and Macek et al., 1969), activated carbon, when based on total endrin (Brungs et al., 1966), and loading density in static tests (Katz and Chadwick, 1961).

[b] Maximum mean interaction = LC50 (mean) at the level of interacting (ambient) factor producing least toxicity (highest LC50) divided by LC50 at the level of interacting factor producing highest toxicity (lowest LC50). Exposure concentrations (ug endrin/l water) were not verified by chemical analysis in all studies cited. A direct comparison between the results obtained within, but not between, separate studies is possible due to experimental design, i.e., the ambient factors examined seem to be the only or main variables.

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